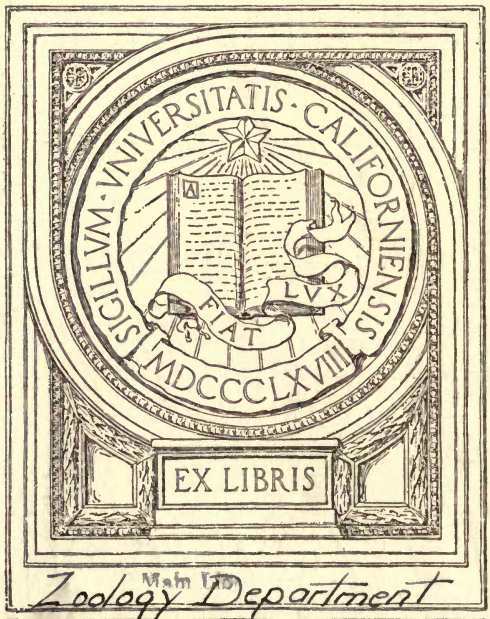
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Laboratory Directions in Principles of Animal Biology

A. Franklin Shull



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IN
PRINCIPLES OF ANIMAL BIOLOGY

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LABORATORY DIRECTIONS

IN

PRINCIPLES OF ANIMAL BIOLOGY

BY

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To

Our Beginning Students

FOR WHOM A KNOWLEDGE OF PRINCIPLES AFFORDS, IN OUR OPINION, THE BEST APPROACH TO ANY SCIENCE

403321

PREFACE

The course for which this book of laboratory directions was prepared is a recognition of the growth which the science of Zoölogy has made in the past several decades. No longer a purely morphological subject, zoölogy is not in the opinion of the authors properly treated in a purely morphological course. Good teachers have long recognized that dissection and classification alone would not make a zoölogist, and have striven in lectures and recitations to provide the larger outlook which the science has come to possess. But this recognition seems hardly adequate. If in the lectures and recitations due attention is paid to the type dissections in the laboratory, morphology can scarcely avoid receiving an emphasis it does not deserve. If to avoid this over-emphasis the recitations and lectures are devoted exclusively to evolution, distribution, ecology, genetics, etc., the laboratory exercises and recitations must seem unrelated to one another. Recitations and laboratory work thus become two courses which the student pursues simultaneously.

The only solution has appeared to be to make the laboratory work itself bear on the large questions of biology. The laboratory work may thus have a balance of its own, it does not need to be averaged with the recitations. This book contains directions for first-hand exercises which we believe have the emphasis properly placed. Morphology still receives more attention than any other division of the subject, but it is nearly everywhere directed to some end which is not merely structure.

The large number of inquiries received concerning this course, indicating a widespread belief that some plan of this kind is preferable to the usual type course, have led us to make the book available for use in other institutions. Many details of the course may well be altered. One form will often illustrate a point as well as another form. In a number of instances alternative tasks are provided; others will occur to the experienced teacher.

In the preparation of the laboratory directions every member of the Zoölogy Department of the University of Michigan has had a share, either in original organization or subsequent revision. Besides those mentioned on the title page as authors, special mention is due to Professors Jacob Reighard, E. C. Case, R. W. Hegner, and Paul S. Welch, and Mr. George E. Johnson.

A. Franklin Shull.

August, 1919.

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LABORATORY DIRECTIONS

IN

PRINCIPLES OF ANIMAL BIOLOGY

A. INSTRUMENTS, SUPPLIES, AND TEXT-BOOKS

Students electing the course in Principles of Animal Biology should furnish themselves with the following:

1 "Principles of Animal Biology" (by Shull, LaRue and Ruthven).
1 "Laboratory Directions in Principles of Animal Biology."
2 Teasing Needles.
½ lb. note paper, 2 doz. drawing sheets, 1 piece press board, all in strong manila envelope.
1 Pair Fine Forceps, milled tips, 5 inch.
1 Millimeter Rule.
3 Medicine Droppers.
½ doz. Slides.
2 doz. ¾-inch Cover Glasses No. 2, Square or Circular.
1 Piece Absorbent Cloth for Cleaning Slides and Covers.
1 5H Venus Drawing Pencil.
1 Eraser with Beveled End.
1 Tablet Fine Emery Paper for Sharpening Pencils.
1 Case for Instruments is desirable.
13 Manila Envelopes Printed for Zoölogy.

These instruments and supplies may be obtained in sets at the various dealers.

B. SCHEDULE

An attempt will be made to maintain a definite schedule in the work of the laboratory. Fill in the blanks below, from the notice posted on the bulletin board showing the date for beginning each subject and the number of periods allowed for it. Plan your work so that you may finish it in the time allotted.

Report Number	Subject	Date of Beginning Study	Number of Periods Allowed
1.	Relation of object and image		
2.	The cell		
3.	Activities of protoplasm		
4.	Mitosis		
5.	Cell aggregation, differentiation, division of labor		
6.	Reproduction		
7.	Breeding habits of vertebrate animals		
8.	Embryology of typical animals		
9.	Homology		
10.	Taxonomy		
11.	Ecology and adaptation		
12.	Zoögeography		
13.	Paleontology		

C. LABORATORY ARRANGEMENTS AND REGULATIONS

1. **General Laboratory Directions.**—Each student on leaving the laboratory should leave his place and instruments in good condition. Return the microscope, dissecting microscope, trays, etc., in good condition to their proper places. Clean your place at the table. Push the stool under the table. Do your part to leave the laboratory in good order for the next section.

2. **Laboratory Notes Must not be Removed from the Laboratory Without Permission.**—At the conclusion of each laboratory period put *all your work, complete or incomplete,* in an envelope, properly labeled and deposit it in the place indicated by the instructor.

3. Always put your name, section and laboratory seat number on your text-book, laboratory manual, and envelopes so that they may be easily identified.

4. Report immediately to the instructor or his assistant any part of the laboratory equipment that is missing or out of repair.

D. LABORATORY RECORDS

The laboratory records consist of notes and drawings which should supplement each other. If laboratory records are to be accurate they must be recorded at the time the observations are made, not hours or days afterward.

1. Notes.—The laboratory work of the course consists of 13 exercises. With the exception of the first, which is a preliminary drill in the use of the microscope, each exercise is designed to illustrate certain generalizations. The facts upon which these generalizations are based can, in

many cases, best be recorded in the form of notes. Such notes, recorded while the observations are being made, will usually be isolated statements, often without connection with those that precede or follow. How frequently such notes should be made is left to the judgment of the student. They are intended solely as an aid to the memory. Obviously, therefore, these disconnected notes need not repeat statements made in the laboratory directions. Likewise, it is superfluous to write in the notes what the drawings show equally well. Unless called for by the instructor, these notes need not be handed in for inspection.

2. Summary.—When an exercise is completed, with his notes and drawings before him, the student should be able to draw certain conclusions from them, or to state the principles which they illustrate. In most of the exercises, those conclusions or principles will be capable of clear expression in the form of a summary. If the student is in doubt as to what this summary should contain, it probably means that he has not grasped the significance of the exercise, and he should ask help. However, not all the exercises lend themselves equally well to recapitulation, and the instructor may indicate, in connection with each one, whether a summary is expected. When a summary is written, it is to be handed in with the drawings for inspection.

3. Drawings form a very essential part of the laboratory records. They should therefore accurately fulfill the purpose for which they are made. Many of them must be *detailed*, not caricatures of the general appearance of the object; when detail is desired frequent comparisons of drawing and object must be made *during the process of drawing*. Drawings should in all cases be analytical, that is, should represent the student's analysis of the structures seen. They should, therefore, be made directly from the specimens themselves. Laboratory drawings should not be considered from the standpoint of art, but from the standpoint of faithful analysis. Sometimes brief *sketches* will suffice to illustrate a specific point; but even these must not be careless.

Special training in drawing is not presupposed, but any student can attend to certain features. Always use a sharp, hard pencil. Very lightly mark in the outlines and general features of the object to be drawn, erasing and redrawing any parts which are out of proportion or incorrect. Then carefully retrace the corrected outline leaving a clean, sharp, single line. Leave no thick lines, nor double lines, nor loose ends, nor gaps between the ends of lines where they do not belong. Draw even small granules with complete outlines and of the proper shape and relative size. If granules are actually irregular make them so. Remember that even minor errors offend the eye.

Make drawings large enough to show the required details.

Shade sparingly, and always with a definite purpose in view. Shading is rarely needed. An excellent method of shading for scientific purposes

is by the use of fine regularly placed dots (stippling). In pencil work, an artist's tool called a blender may be used to secure an even gradation of shading.

4. Drawings and Legend.—The pages or "Plates" of drawings should be numbered consecutively through the course with Roman numerals, in the upper right-hand corner (Plate I, Plate II, etc.). The student's name should appear in the upper left-hand corner. The individual structures in a drawing may be labeled with the initial letter (or first two letters) of their names, and an explanatory legend be placed at the bottom of the plate; or, if preferred, the entire names may be written beside the figure. In the latter case, the labeling must be neat and so inconspicuous as not to overshadow the drawing. If the drawing is done with a pencil, the labeling should be done with a pencil. A neat style of lettering for free-hand work is shown in Fig. 1. Refer to it if in doubt as to the correct form of letters.

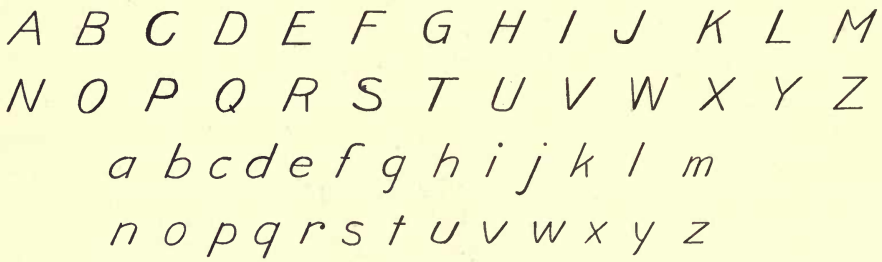


FIG. 1.—The alphabet in suitable style for free-hand lettering.

Do not crowd the drawings on the plate but plan the plate so that there will be ample room for the drawings, the lettering, and the legend. Always take a fresh sheet of drawing paper when beginning a new exercise, so that drawings of two different exercises will not be found on one sheet.

E. FILING AND CORRECTION OF THE REPORT

At the conclusion of each laboratory period place all notes and drawings, finished or unfinished, in an envelope, fill in the blanks on the face of the envelope, and file the report in the place designated by the instructor. Notes and drawings must not be removed from the laboratory except by permission. They must be available for inspection at all times except when the student is actually working on them. At the time indicated by the instructor for the completion of the study on each exercise the report will be taken up, graded, and returned. The student, while in the laboratory, should make the corrections indicated and then place the work in an envelope where it is to be kept until the end of the semester. At that time the notes and drawings, arranged in proper order, must be returned to the laboratory for inspection.

EXERCISE I

USE OF THE MICROSCOPE

Before beginning work, it is necessary to become familiar with the microscope and the method of using it. The first laboratory period will be devoted to this.

Identify the stand, the oculars, the tube, the objectives, the stage, the diaphragm, the mirror, the foot, coarse adjustment, fine adjustment, clips. Study the illustration, Fig. 2, for other features.

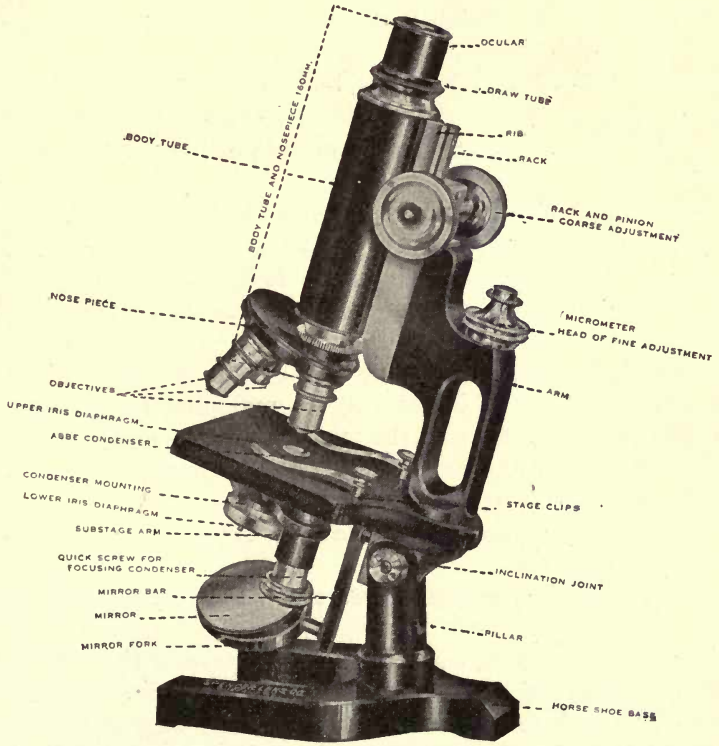


FIG. 2.—A modern microscope, with its parts named. (*Courtesy Spencer Lens Co.*)

Understand perfectly how to change from "low power" to "high power," and the reverse; also which direction to turn the coarse adjustment and the fine adjustment to raise or to lower the body tube.

In using the microscope, note especially the following points:

1. *Never focus downward while looking into the microscope* as there is great danger thus of driving the objective against the object examined, to the great injury of both.

2. Never wipe off the ocular or objective with handkerchief, cloth, or anything except *lens paper*, which will be furnished as needed.

3. In case the ocular or objective cannot be readily cleaned or is injured in any way take it at once to one of the instructors. Do not try to clean it yourself.

5. Report *at once* to the instructor any missing parts or injuries to the microscope.

PREPARED SLIDE OF A PRINTED LETTER

1. Focusing.—Place the 4x ocular and 16 mm. objective in position and adjust the mirror so that the light from the window passes up through the tube of the microscope. Now so place the slide of the printed letter on the microscope that its label may be read (that is, right side up) and the letter is as nearly as possible in the center of the aperture in the stage.

Lower the tube of the microscope by means of the coarse adjustment until the objective almost touches the cover-glass; then with the eye at the ocular slowly move the tube *upward* until the letter on the slide appears distinct.

2. Relation of Object and Image.—With the slide held as in (1) make a *drawing* (on drawing paper) of the letter as seen with the unaided eye, and another *drawing* of the image made by the microscope. Before making your drawing, refer again to the instructions for labeling drawings and plates.

These drawings should be made of the same size as the image and object respectively. The image may be measured by laying a millimeter scale across the stage of the microscope at one side, and looking into the microscope with one eye and at the scale with the other. The scale will appear to lie over the image.

This sheet of drawings is your Plate I. Always follow this style in making up your plates.

Now using note paper and ink state how the image differs from the object.

3. Illumination.—Note carefully the brightness of the field of vision and the appearance of the letter; it is illuminated by transmitted light. Tilt the mirror and observe the change in the intensity and character of the light. The object is now viewed by reflected light which must be employed for all opaque objects.

4. Magnification.—Determine what combination of ocular and objective gives the lowest magnification, what combination the highest, etc. Make a *table* showing all the combinations of objectives and oculars arranged in the order of their magnifying power.

The student should note that the oculars are marked to denote their magnification, thus, 8x and 4x, while the objectives are marked in terms of their focal length, thus, 4 mm. and 16 mm. These terms should always be used in designating the oculars and objectives rather than the expressions high power and low power, or large and small.

5. Other objects will be furnished for examination.

Note to the Student.—The work of this laboratory period constitutes report number 1. Place all the notes and the plate (see that each sheet of your work has your name on it) in an envelope, fill in the blanks giving as the subject of this report "The Relation of Object and Image," and put the envelope in the place indicated by the instructor. Put everything away in good order. Put waste paper in the waste basket; push the chair or stool under the table; leave the table in as good condition as you would like to find it. Take this book of laboratory directions home with you and study carefully the general laboratory regulations, the statements concerning the laboratory records, and the use of the microscope.

Be ready for a quiz at any time on the work completed.

EXERCISE II

THE CELL

It is the purpose of this exercise by means of a study of actual materials to acquaint the student with the general facts in regard to the structure of the cell and the extent to which the cell occurs as a unit of structure in living things. In order to accomplish this object a general problem is stated and this general problem is subdivided into minor problems. Appropriate materials with suggestions for study are given under each subdivision.

At the conclusion of the exercise the student should be able to formulate certain inferences in regard to the cell. He should realize that the study of the relatively small number of materials suggested in the course of this exercise do not furnish data sufficient for the confirmation of the cell doctrine but that the facts observed belong to certain classes of facts on which the modern cell doctrine is based.

A. THE GENERAL PROBLEM

To determine the structure of the cell and the extent of its occurrence as a unit of structure in living things.

1. What Structural Features are Common to Cells?

A complete answer to this problem cannot be made until all the materials in this exercise have been studied but a study of cells from the four sources indicated below (1*a*, 1*b*, 1*c*, 1*d*) will serve to introduce certain structures and will give an idea as to the forms which a cell may assume.

1*a*. Place a drop of water on a clean slide and mount in it a small piece of stratum corneum of frog skin (the outermost layer that is repeatedly shed). Spread the specimen flat, and cover with a cover-glass. Examine with the microscope, trying out different light intensities.

Note the units of which the tissue is composed. These are the *cells*. Each contains a dense mass, the *nucleus* (plural, *nuclei*), which is usually visible. The remainder of the contents of the cell, besides the nucleus, is to all appearances nearly structureless and is known as *cytoplasm*. Both nucleus and cytoplasm are composed of protoplasm. The surface layer of each of these cells is the *cell membrane*.

Now remove the cover-glass or mount a fresh piece of stratum corneum. Draw off the excess water with filter paper or a blotter, and add a

drop of erythrosin, which is a staining solution. After half a minute remove the surplus stain, add a drop of distilled water, and put on a cover-glass. Which part of the cell is most intensely stained?

Draw a group of three or four cells, each one about half an inch in diameter. An outline of the cells and their nuclei will suffice, but it should be neat. Label nuclei and cytoplasm.

1*b*. Examine a section of the liver of a frog or salamander which you will find in the tray on the table. Is this made up of cells? What part of the cell is most intensely stained? Is a nucleus found in each cell? If not, explain its apparent absence.

1*c*. Examine a slide of stained snake, bird, or salamander blood. *Draw* an oval corpuscle in outline, showing the nucleus. This is one of the corpuscles which gives the blood its red color but its present red color is due to the fact that it has been stained.

1*d*. From a culture containing Protozoa (one-celled organisms) mount a drop of water. Before putting on the cover-glass, examine the slide with low power to see that the organisms are present. Then add a drop of acetic methyl-green, mixing the stain with the drop containing Protozoa, and put on the cover-glass.

This solution kills the organisms and stains the nucleus of each. How many kinds of nucleated organisms do you find?

In the cells studied thus far the nucleus, cytoplasm and cell membrane have been demonstrated. During the remainder of this study note carefully whether these cell structures are present in the cells studied.

2. What are Some Other Structures Found in Cells?

2*a*. Examine a leaf from the growing tip of Elodea. Note the cell *wall* which limits the protoplasm of the cell. Is this cell wall relatively thick or thin? *Draw* the cell wall of a single cell showing also the connections with the walls of neighboring cells. The figure should be 1½ to 2 inches long.

Do all cells have enveloping structures such as cell walls or cell membranes? Reserve the answer to this question until you have examined the cells in the remainder of this study and include your answer, which should be in some detail, in the summary.

2*b*. Examine Euglena or other green flagellate for colored bodies. Mount in water a bright green leaf of Elodea taken from a growing tip of a branch and examine it for colored bodies. These are *plastids*. Search in Elodea for plastids shaped like two biscuits fastened together. How do you explain this shape? Color of fruits and of many flowers may be due wholly or in part to the presence of colored plastids. *Chromoplast* is a general name for all colored plastids while the word *chloroplast* is used to designate only green plastids.

Draw in outline one cell with its plastids. If Elodea is chosen for

this sketch, the chloroplasts may be added to the figure of the cell wall already drawn. In this case plastids shaped like two biscuits fastened together should be included if they are found. Be on the lookout for plastids in other unstained cells to be studied later.

2*c*. In mounted sections of Hydra study the cells of the innermost layer. Hydra is a small many celled animal, having certain affinities with jelly fishes, corals, sea-anemones and hydroids. Its body is composed of two distinct layers of cells separated by a non-cellular layer. Note particularly the large clear spaces within the inner cells. These spaces are *vacuoles*. *Draw* in detail a group of three cells of this layer showing structures present.

2*d*. Examine specimens or Phacus or Euglena (both green flagellate Protozoa) for *paramylum* bodies. These are granules of stored food, resembling starch in its chemical composition. They are colorless. Their shape differs in various species, being discoid in some, ring-shaped, rod-shaped, or polyhedral in others. If Euglena is used for this study flatten the specimen by withdrawing water from the preparation and look for minute colorless bodies among the green. Do not mistake the rounded nucleus near the middle of the body and the reservoir of the contractile vacuoles near the anterior end for the paramylum bodies.

2*e*. Remove a frog's egg from its jelly-like covering, then tease out (tear up finely with needles in water) the substance of the egg upon a slide, separating the particles until they form a very thin layer on the slide, and mount under a cover-glass. Examine with the microscope. The fine granules are yolk material (stored food). *Sketch* a group of them.

3. What are Some of the Structures of the Nucleus?

3*a*. In longitudinal sections of a dorsal root ganglion (a small mass of nervous tissue occurring near the junction of spinal nerves with the spinal cord) of a cat look for a single rounded body near the center of many of the nuclei. This is the *nucleolus*. Note its color. Observe the *chromatin* which occurs as granules in the nucleus. Compare the colors of these granules. *Sketch* in detail a cell of the ganglion to show nucleolus or nucleoli and chromatin granules. The sections on these slides were stained with two stains. All parts of each cell were subjected to the same processes. On what basis may the difference in color between the nucleolus and the chromatin granules be explained?

4. What is the Structure of a Simple Living Cell?

Amœba furnishes an example of such a cell.

4*a*. Mount some ooze from a culture containing *Amœba*. The student should endeavor to find a specimen for himself. If an amœba cannot be found ask help, but *do not discard the slide*. It may have Amœba on it, even if the student has not been successful in identifying one.

4*b*. Describe the general appearance of Amœba, its color or lack of color. Be specific.

4*c*. The blunt processes thrust out from the body are *pseudopodia* (singular, *pseudopodium*). Do they change shape or size? If so make three sketches in outline only of the entire amœba at intervals to show these changes. What relation exists between the pseudopodia and the movement of the body as a whole? In some species there is only one pseudopodium.

4*d*. On using high magnification note the outer clear layer of protoplasm, often quite thin; this is the *ectosarc*. Within the ectosarc is the granular *endosarc*. Note the movements within these two layers, especially in the formation of a pseudopodium. Which layer moves more rapidly when free to move? What conclusion may be drawn regarding the relative fluidity of ectosarc and endosarc? Give reasons for the answer to this question.

4*e*. In large specimens vacuoles containing particles of food may be seen. The larger food vacuoles may be recognized by their contents. In which layer are they? What are their contents? These contents are cell inclusions, not part of the cell.

4*f*. Look for one or more pulsating vacuoles. These are not always visible but if the specimen be watched for a few minutes small vacuoles may be seen which increase in size and finally move to the surface where they collapse. Under certain conditions of light the contractile vacuoles may have a slight pinkish cast. The disappearance of the vacuole is one feature that distinguishes the contractile vacuole from the more persistent food vacuoles. The latter also contain food particles.

4*g*. Find the nucleus, a rounded, highly refractive somewhat grayish body, occurring in some species in a vacuole-like structure. Is it in the ectosarc or endosarc? If you do not see the nucleus clearly in your specimen consult the demonstration of a stained specimen.

4*h*. Make a careful *drawing* of Amœba not less than two inches in diameter, showing all the structures noted in the foregoing study. Ask for instructions if in doubt on any point.

If on any point of structure or appearance your drawings fail to give a proper idea, a description should be given also.

5. How May Cells be Modified?

5*a*. *Cartilage Cells.*—On the upper end of the humerus or femur of a preserved frog note the glistening white cap. This is cartilage. Place a drop of water on a slide. Then with a very sharp scalpel or safety razor blade shave off a very small, thin piece of cartilage from the surface, and touch the edge of the blade to the drop of water. Cover, and examine the thin edge of the cartilage with the microscope. How are the cells arranged? Do the cells touch one another? The intervening

substance is the *matrix*. What inference may be made concerning its origin? *Draw* a few groups of cells, showing the structure of at least two of them, and representing also the matrix.

5*b*. *Bone Cells.*—Examine prepared sections of dry bone. The dark spots are the spaces or *lacunæ* (little lakes) formerly occupied by the bone cells. Projecting from the lacunæ are minute wavy channels, the *canaliculi* (little canals) into which in life extend slender processes (like pseudopodia) of the bone cells. Larger openings, for blood vessels, may occur in the preparations. The remainder of the specimen consists of the hard parts, mostly calcium salts, deposited in the matrix. The fleshy parts of the bone are dried and shriveled in these preparations. What is the origin of the matrix?

Draw carefully a lacuna with its canaliculi as representing the form of a bone cell.

5*c*. Remove a hair from your eyebrow, mount it in water and examine at different points along its length both on the margin and on the upper surface. Can you detect anything that would indicate cells? *Sketch* a segment of the hair to show them. In the sketch the diameter of the hair should be one-half inch.

5*d*. *Wool* is similar to hair in its composition. Examine the minute fibers from a woolen blanket. Are there any indications of cellular structure?

5*e*. *Human Blood.*—This exercise is not required. Any one who desires to examine his own blood will be shown how to do so with comparative safety and with the minimum of pain. Mount the blood, and examine the red corpuscles. What is their color when seen singly? Is there a nucleus? Examine a demonstration of stained human blood.

6. Is the Whole Animal Body[1] Made up of Cells?

6*a*. Examine a longitudinal section of a small salamander. The names of most of the structures (organs) which you find there may be determined by consulting the wall chart. Make a list of all the organs which you think are made up of cells? Do you find any organs not made up of cells? If so make a list of them also. If you are in doubt as to the interpretation of any of the observations consult the instructor or the assistant. (The presence of nuclei may be taken as evidence of the existence of cells if the cell outlines cannot be determined.)

6*b*. Recall also in connection with this problem the parts of the animal body studied 1*a*, 1*b*, 1*c*, 2*c*, 3*a*, 4, 5*a*, 5*b*, 5*c*, 5*d*, 5*e*.

B. SUMMARY

In your summary state the general conclusions in regard to the cell which may be derived from the facts presented in this exercise.

[1] To the teacher: By the addition of proper plant material this problem may be made to cover all living things.

EXERCISE III

ACTIVITIES OF PROTOPLASM[1]

Living protoplasm exhibits certain properties which distinguish it from non-living matter. Among these are *independent movement* and *metabolism*. Independent movement is the result of the instability of living substance and its reactiveness to chemical and physical forces. Metabolism includes the taking in of food, its transformation into energy or into more living substance, and the elimination of waste formed during the process.

Only a few of the more easily demonstrated functions of living matter are studied in this exercise. Even a representative series of experiments in physiology would require considerable time, and some previous training in biology and related subjects.

Notes.—Much of the work outlined below is not recorded in drawings Notes should be written with special care in such cases.

A. FUNCTIONS OF THE CELL

Vital phenomena are first studied in the cell since all activities of the protoplasm are fundamentally cell activities.

1. Movements of Protoplasm.

1*a*. Mount a young green leaf of Elodea, recently collected, under a cover-glass. Under high magnification look for movements of the protoplasm inside of some of the cells. If movements are not observed at first they will usually begin after a few minutes. This form of movement is known as "*rotation.*" In what region of the cell does it occur? Note the time required for a complete rotation. Compare the direction of rotation in adjacent cells. *Draw* an outline of the cell and indicate by arrows the direction of rotation.

1*b*. Recall the movements of the protoplasm in the endosarc of Amœba, especially as it enters a newly formed pseudopodium. If material is available this should be observed again. This movement of the protoplasm is known as "streaming."[2]

1*c*. *Amœboid Movements.*—Recall the movements of Amœba by means

[1] To the teacher: It is not essential that all of the experiments and observations outlined in this exercise be employed. The ones to be selected may depend in part upon the amount of time to be devoted to the subject, and upon the facilities of the laboratory.

[2] At the option of the instructor movements like those mentioned in 1*a* and 1*b* may be observed in Paramecium, in hairs from the stem of the tomato plant, in stamen hairs of Tradescantia, or in some other plant.

of blunt pseudopodial processes. Such movements result in locomotion or the engulfing of food.

1*d*. *Ciliary Movements.*—Paramecium. Examine specimens of Paramecium mounted on a slide. Note their movements. Introduce a small drop of iodine along the edge of the cover-glass. The iodine kills the animals and stains the cilia covering their surface. Study these cilia. Approximately how much of the cell is devoted to ciliary movements? Determine whether the whole surface is covered by cilia. Are the cilia like the rest of the protoplasm or may they be regarded as specialized for movement?

Rotifers. Place a number of rotifers (wheel animalcules) on a slide and examine with a microscope. Observe the ciliary movements at the broader end of the body. Describe the structure and arrangement of the cilia. Do they beat with equal vigor in both directions, or more strongly in one direction? If the latter, is the form of the cilium during the stronger beat the same as during the weaker one? If you detect a difference, make a *drawing* of a single cilium to illustrate the difference.

Gill of a freshwater mussel.[1] Mount a small piece cut from the edge of the gill of a freshwater mussel. The epithelium covering the gill is ciliated. As the piece slowly dies, the movements of the cilia diminish. Observe their movements as in the rotifer.

1*e*. *Flagellate Movements.*—Examine Euglena or Peranema on a slide and note its form of locomotion. Introduce some iodine along the edge of the cover-glass. Now look for specimens which have been killed by the iodine. A long whiplike thread at one end is the flagellum by means of which the animal moves. Flagellate movements are less common than ciliary movements in tissues of higher forms.

In which, if any, of the specimens so far studied is the moving protoplasm like the quiet protoplasm near it? In which, if any, does the moving protoplasm appear to be differentiated for movement?

2. Metabolism.

2*a*. *Ingestion.*—Mount some paramecia on a slide and note the color of the round bodies (*food vacuoles*) in them. Put a small drop of carmine suspension (well shaken) along the side of the cover-glass. (India ink may be substituted for the carmine.) After a moment note the formation of red vacuoles inside of the animal. The small carmine particles have been ingested. Study a chart or model of Paramecium and determine how ingestion is accomplished.

2*b*. *Secretion and Digestion.*—Find a specimen on the above slide that is quiet. To quiet them it may be necessary to press down the cover-glass slightly or put them into a jelly made by steeping crushed quince seeds in

[1] The gill of a freshwater mussel may be used as a substitute for the rotifers or in addition, at the option of the instructor.

water. Examine with high magnification and determine the structure of the food vacuoles. What proportion of the vacuole is liquid and what proportion is solid material? Where are the food vacuoles found? What accounts for their distribution? Are the carmine vacuoles evenly distributed? If not where are they most abundant? Do you observe any difference in the various food vacuoles? What may be the object of the liquid in the vacuoles? What may be its source?

Neutral red gives an opportunity to determine the nature of some of the chemical processes taking place in the vacuoles since it stains acid substances red and alkaline ones yellow. To prove this put a drop of weak alkaline solution (NaOH) and another drop of weak acid solution (HCl) at opposite ends of a slide resting on a white background. Add a drop of neutral red (0.001 per cent. solution) to each of the drops on the slide and note results.

Mount a fresh slide of paramecia and put a drop of neutral red along the edge of the cover-glass. This will reach the paramecia after a few minutes, with the usual result that some of the food vacuoles are stained red, others will be found to be colorless, and still others will have a pale yellowish tinge. What does this suggest as to the nature of the contents of the three kinds of vacuoles? What is the source of the substances indicated in the vacuoles? Other substances whose action may account for the digestion of the food in the vacuoles may be inferred at the end of the exercise.

2*c*. *Absorption.*—After food is rendered soluble by digestion it is absorbed by the surrounding protoplasm. This process cannot readily be demonstrated. How may it take place in Paramecium?

2*d*. *Respiration and Oxidation.*—This function is also difficult to demonstrate in single cells. In Paramecium oxygen is taken in directly through the surface of the body and carbon dioxide is given off in the same way. What provision, if any, is made for bringing in oxygen to the various parts of the body and for taking carbon dioxide out to the surface? How is a constant supply of fresh water brought to the animal?

2*e*. *Excretion.*—Excretion takes place in Paramecium by means of two clear *pulsating vacuoles.* Mount some paramecia and observe the vacuoles in a quiet specimen. Note that they increase in size and disappear at intervals. Where are they located? Observe the *radiating canals* around them. At what stage in the pulsation of the vacuoles are these canals most conspicuous? What do the vacuoles contain? What is the relation of the canals to the vacuoles? What becomes of the contents of the vacuoles when they disappear?

B. FUNCTIONS OF TISSUES AND ORGANS

The cell in one-celled organisms has a generalized function. In multicellular organisms different cells have become specialized to perform

different functions. Cells having similar functions combine to form *tissues* and tissues unite to form *organs* of various kinds.

3. Movements.—Movements in higher animals are usually due to the concerted action of numerous specialized cells known as *muscle cells.*

3*a*. Examine a longitudinal section of the tail of a young salamander. A considerable portion of the section passes through the muscles. Note that the muscle mass is divided into segments, the *myotomes,* by oblique *septa* of connective tissue. Muscle cells extend lengthwise from one septum to the next. What is the relative length and width of each cell? (The width can best be determined near the septa.) How many nuclei in one cell? Observe the longitudinal threads or *fibrils* in the cells. Note the transverse light and dark bands on the fibrils. These are known as *striations.* Are the striations continuous lines across each cell?

3*b*. If time permits a bit of teased out preserved frog muscle should be examined for the structures found in the above preparation. An oil immersion demonstration will also be provided showing the structures more in detail.

Muscular movements are due to changes in the muscle fibrils resulting from a change in the relative size and shape of the light and dark bands. The process is not very well understood. Approximately what fraction of the muscle cell is given over to the function of movement? Is the movement performed by the general protoplasm or by specialized structures?

3*c*. A muscle removed from the body will respond to various forms of stimuli such as *mechanical, thermal, chemical,* and *electrical.* The last is usually employed in laboratory experiments. The contraction of a muscle will be demonstrated by the following experiment:

The gastrocnemius muscle of a frog is removed and suspended by means of a clamp attached to the leg bone. A small weight is attached to the lower end by means of a hook. The muscle should be kept moist with normal salt solution. Touch the muscle with platinum electrodes attached to a dry cell. Note the contraction. Is the movement slow or rapid? How does the muscle change in shape? How much does it shorten? When does the contraction occur, at the application of the stimulus, during the passage of the current through the muscle, or at the removal of the stimulus?

3*d*. In the body the muscle usually responds to stimuli that come to it through a nerve. The conduction of an impulse through a nerve may be demonstrated by a nerve-muscle preparation of the gastrocnemius of the frog. In such a preparation the nerve going to the muscle is left intact. Apply the stimulus to the nerve some distance from the muscle and compare the contractions with those above.

4. Metabolism.

4*a*. *Ingestion* takes place in higher animals through the mouth.

4*b*. *Secretion and Digestion.*—Digestive juices and *enzymes* are secreted by specialized cells which often unite to form *glands*. Examine the cross-section of the stomach of a frog. Note the layer of cells, the *mucosa*, lining the interior. Note the elongated nucleus near the base of each cell. At frequent intervals the mucosa dips down into the underlying tissue in the form of slender tube-like pits. These pits are the *glands*. Find a gland which is cut throughout its whole length. At some depth in it note a group of clear vacuolated cells. If the section is cut from the anterior (cardiac) end of the stomach, the gland will extend much deeper than the group of clear cells. The gland is everywhere composed of a single layer of cells around a slender open canal. *Draw* (without stippling) a stomach gland.

The glands of the stomach secrete *hydrochloric acid* and *pepsin*. Test the action of these as follows: Place a small piece (half as large as a pea) of hard-boiled white of egg into each of three test-tubes or dishes, taking care to make the pieces of equal size. To one tube add 10 cc. of a 0.2 per cent. solution of hydrochloric acid (2 cc. of the acid to a liter of water); to another 10 cc. of a solution of pepsin in water (1 gram of pepsin to a liter of water); to the third 10 cc. of a solution of pepsin in 0.2 per cent. hydrochloric acid. Put all the tubes into a water bath or incubator, and keep at a temperature of 40°C. Observe the three tubes at the end of the laboratory period, and daily thereafter. What conclusion do you draw from the experiment?

Among other digestive glands found in higher animals may be mentioned the *salivary glands* opening into the mouth, the *pancreas* opening into the intestine, and the *intestinal glands* in the walls of the intestine. The enzymes secreted by these glands digest the various kinds of foods, namely, *proteins*, *carbohydrates*, and *fats*.

4*c*. *Absorption.*—Absorption is principally an osmotic phenomenon. *Osmosis* may be briefly defined as the passage of water and dissolved substances through a permeable membrane. If the membrane separates two liquids of unequal density, the greater flow is toward the liquid of greater density. Osmosis may be illustrated by the following demonstration experiment: Tie a wet piece of parchment paper or animal bladder over the end of a thistle tube. Fill the tube with a concentrated solution of copper sulphate and support it in a beaker of water so that the level of the water and the copper sulphate is the same. Examine at the end of the laboratory period and also at succeeding laboratory periods. Observe the results and explain.

The intestine and blood vessels are lined with permeable membranes through which osmosis takes place in a similar manner.

4*d*. *Circulation.*—This is accomplished in higher animals by the

blood system. Study the beating of a frog's heart in a demonstration specimen. Also observe the circulation of blood in the blood vessels of the web of a frog's foot. What are the formed objects in the blood? Observe the thinness of the blood vessel walls. With what are they in contact outside? Note differences in the size of the blood vessels. Does the blood flow in a steady stream in all the vessels? Why?

4*e*. *Respiration and Oxidation.*—Oxidation with the liberation of energy takes place in the tissues. The oxygen needed in the process is supplied through the lungs in higher forms and usually through gills in lower forms of animals. Carbon dioxide which is formed during the process is eliminated through the same organs. Presence of carbon dioxide in the expired air may be demonstrated by the following experiment which can be performed by each student. Put a little lime water, $Ca(OH)_2$, in a test-tube and blow through it with a glass tube or blow pipe. Note results. The CO_2 in the expired air combines with the $Ca(OH)_2$ to form an insoluble substance calcium carbonate, $CaCO_3$. Now pass some ordinary laboratory air through a fresh supply of lime water by means of a large rubber bulb and a glass tube. Results? Conclusion?

4*f*. *Excretion.*—Most of the nitrogenous waste products are removed by the *kidneys*. Study a cross-section of the kidney of a frog. The kidney is made up of small tubes, much coiled, and the section cuts these tubes at all possible angles. Note that the tubules are more distinct in some parts of the section than in others. From a chart learn the arrangement of the tubes and their connection with the ducts leading from the kidney.

In one part of the section find a number of rounded bodies, the *glomeruli*. These lie within small capsules known as *Bowman's capsules* at the end of the tubules. These are difficult to make out in the sections and a chart should be consulted. A Bowman's capsule and its glomerulus are together known as a *Malpighian corpuscle*. The glomeruli are coiled blood vessels, and the yellowish cells in them are red blood cells. Find red blood cells elsewhere in the section, outside of the glomeruli. In a demonstration specimen of an injected kidney note again the glomeruli and the numerous blood vessels in the rest of the kidney. The tubules are not easily made out in this section. What function may the close proximity of tubules and blood vessels serve? What physical phenomenon may account for the elimination of waste by the tubules of the kidney?

From your reading be sure you understand the main facts of the structure of the kidney and the functions of the kidney.

C. SUMMARY

In your summary of the functions of protoplasm compare the functions of one-celled animals and of other single cells with the functions

of many-celled animals. What functions have been studied in each? Is coöperation or division of labor involved in either case? The guide questions throughout the exercise will suggest valuable comparisons.

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EXERCISE IV

MITOSIS (KARYOKINESIS)

Also Called

INDIRECT CELL DIVISION

A. INTRODUCTION

In this type of cell division characteristic changes occur in the nucleus, the cytoplasm, and the centrosome. The most important changes take place in the *chromatin*, the deeply staining portion of the nucleus. The nuclear membrane disappears, and the chromatin, which was arranged in a net-like reticulum, gradually rearranges itself into fine coiled threads (*fine spireme*) which shorten and thicken into coarser, more loosely coiled threads (*coarse spireme*). From the coarser threads are developed, by further shortening and thickening, definite bodies called *chromosomes*. The number of chromosomes is different for different species, and is constant for any given species.

In the cytoplasm a spindle-shaped figure composed of thread-like structures is formed. At the ends of the spindle, where the threads converge, are two deeply staining bodies, the *centrosomes*, from which other threads radiate in all directions. These latter radiating threads are the *astral rays*. Upon the middle of this spindle the chromosomes take their place in a flattened group, the *equatorial plate*. Each chromosome splits longitudinally and equally and the two halves go to opposite ends of the spindle. Thus two new groups of chromosomes are formed, each of the same number as was present in the group from which they came. A cell membrane forms between the groups of chromosomes, dividing the cell into two cells. The chromosomes of each group now undergo a series of changes approximately the reverse of those in the early stages of division; that is, they become diffuse again, spinning out into a fine reticulum, thereby forming two new nuclei like the original one.

For convenience the process of mitosis may be divided into four intergrading stages: (1) the *prophases*, that is the early stages up to and including the equatorial plate; (2) the *metaphase*, in which the chromosomes divide longitudinally; (3) the *anaphases*, in which the half chromosomes are distributed to opposite ends of the spindle; and (4) the *telophases* which include the division of the body of the mother cell and the reconstruction of the daughter nuclei.

B. MITOSIS IN THE SEGMENTING EGG OF ASCARIS

Mitosis may be readily studied in the segmenting egg of *Ascaris megalocephala* (a round worm parasitic in the intestine of the horse), in the skin of young salamander larvæ, or less satisfactorily in the segmenting egg of the white-fish. The description below applies directly to Ascaris, but may be modified to apply to the others.

A knowledge of the nature of the specimens of Ascaris in which mitosis is studied will obviate some confusion. The salient features follow:

(*a*) The segmenting eggs are in the uterus, a tubular organ, which is cut in thin sections. If the sections are cut longitudinally, each ribbon shows the walls of the uterus at the edges, with the eggs between.

(*b*) The eggs have been fertilized, so that in the earliest stages two nuclei (egg nucleus and sperm nucleus) are present.

(*c*) The eggs are turned in all possible positions, so that only one, or both of the nuclei may show, also the later division figures may be observed in various positions.

(*d*) Each section includes only fractions of the eggs, so that only portions of the nuclei or spindles may be present, or these structures may be wholly lacking.

(*e*) After the first division of the egg, only certain of the cells divide in the same manner as the first segmentation. The directions below apply to the first division and later ones of the same kind.

Directions for Study

1. Resting Nucleus.—Study a cell not undergoing division. Note the *nuclear membrane;* the net-like arrangement of the chromatin in the nucleus; and one or more *net knots* (thickenings in the chromatin network). How is the chromatin distributed through the nucleus? What is the appearance of the cytoplasm?

Draw a cell with resting nucleus, showing also the nature of the cytoplasm. The thick membrane around the egg, and at some distance from the egg may be omitted.

2. Prophases.

2*a*. Find a cell in which the chromatin is arranged in distinct but still slender threads (*fine spireme*). Where are these found in the nucleus? Observe the nature of the cytoplasm. Look for a darker mass, the *attraction-sphere,* in the cytoplasm. A dark central granule may or may not be visible in this mass.

2*b*. Select a cell in which the chromatin is in thick worm-like strands (*coarse spireme*). Is the nuclear membrane still present? If so, where in the nucleus are these strands? Observe the cytoplasm. The attraction-sphere may be divided into two parts near together, each

part with lines radiating in all directions into the cytoplasm. Each part is an *aster*. The two asters may be connected by other lines, the double structure being the *amphiaster*. If only one aster is present, how do you account for the absence of the other? *Draw a* or *b*.

2*c*. Find cells in which the nuclear membrane has disappeared, and in which the chromosomes, now quite thick and distinct, have no definite arrangement. Count the chromosomes. Look in the cytoplasm for an amphiaster. Each aster should contain a central granule, the *centrosome*. If only one aster is seen, where is the other? *Draw*.

2*d*. In a later stage the chromosomes are arranged in a flat group. Seen on edge, they form a nearly straight line; viewed from the flat side of the group, the chromosomes are readily distinguishable. How many are there? This group of chromosomes is the *equatorial plate*. In a cell in which the equatorial plate is seen on edge, note the two attraction-spheres and centrosomes (that is, observe the amphiaster). The lines connecting the asters with the chromosomes and with each other are called *spindle fibers*.

Draw a cell with the spindle in side view; that is, with the equatorial plate seen on edge. *Draw* another cell to show the equatorial plate as viewed from one of the centrosomes.

3. Metaphase.—While in the equatorial plate, or earlier, the chromosomes split longitudinally. If you do not find this stage readily, ask for a demonstration either in Ascaris, or in the skin of a salamander, or in some other cell.

4. Anaphases.—Find cells in which the halves of the divided chromosomes have begun to separate into two groups of chromosomes. If possible, count the chromosomes in each group. Note the form of the spindle. Are there fibers between the groups of chromosomes? *Draw* either an early or a late stage; that is, one in which the groups of chromosomes are still near together or are widely separated.

5. Telophases.—Search for a later stage than 4, showing the two groups of daughter chromosomes separated by a cell membrane which has divided the original cell into two cells. Is the nuclear membrane present around the groups of chromosomes? Are the centrosomes visible? Do any signs of the spindle remain? *Draw*.

C. SUMMARY

Give a brief but clear account of the whole process of mitosis. Do not treat it as a series of stages, like the ones you have studied, but as a continuous process. That is, fill in the gaps between the stages studied, using any reliable source of information.

EXERCISE V

CELL AGGREGATION, DIFFERENTIATION, AND DIVISION OF LABOR

A. AGGREGATIONS OF CELLS

When a unicellular animal divides, two daughter animals are formed which usually separate from one another. Thus one-celled organisms are always of small size, in most cases invisible to the unaided eye. Animals that reach visible dimensions almost always consist of more than one cell. Increase in size, in these, is due to accumulation of the cells as they divide. A group of cells derived from one cell by division may be called a *cell aggregation*. Various types of aggregation are described below. Try to discover their fundamental differences while this study is in progress, and arrange them in a definite scheme at the end of the exercise.

1. Epistylis is a colonial protozoön usually found attached to small freshwater animals. Examine demonstrations of stained specimens. Note the method of branching. The oval-shaped bodies at the ends of the branches are the individuals of the colony, and each one consists of a single cell. Note the *nucleus;* what is its shape? Are the cells alike, or distinctly different?

Reproduction in Epistylis takes place by a simple division of an individual into two daughter individuals which remain attached to the colony by independent stalks.

Sketch a small colony.

2. Carchesium and Zoothamnium are other colonial Protozoa. Observe living specimens if obtainable, otherwise omit this section. Study a colony in a salt cellar with a dissecting microscope. Note that each individual is attached to the end of a long contractile *filament* or *stalk*. Can it retract itself independently of its fellows? The contractile element is absent in Epistylis. Note the result of touching one or several individuals with the point of a needle.

In Carchesium and Zoothamnium as in Epistylis the cells are independent of each other and each cell elaborates its own stalk, and carries on the metabolic processes, movements and reproductive functions independently of the colony as a whole.

Write out your observations on the living Carchesium.

3. Pleodorina californica is a free-swimming organism found in freshwater ponds. Study preparations of stained specimens. Note the small

spherical bodies. How many cells do they contain? How are they held together? Note that the cells on one side are larger than those on the other side. The large cells are the reproductive or *germ* cells; the small ones are sterile, and are called *somatic* (body) cells. How many kinds of somatic cells are there? How many kinds of germ cells?

Note if possible the slender whip-like structures projecting out from each cell. These are the *flagella*, organs of locomotion. Do they project beyond the jelly? Would movement of these flagella result in movement of the individual cells, or of the whole group? Is the association of the cells more close or less close than in the two preceding forms?

Sketch Pleodorina.

4. Volvox, like Pleodorina, is a free-swimming organism found in fresh-water ponds. Study living specimens if available in a salt cellar with the dissecting microscope. In case preserved material must be used, place a few drops of liquid containing Volvox on a slide, add three to ten grains of fine sand to support the cover-glass and then put on the cover-glass. Compare with Pleodorina as to size, shape and number of cells.

Are the cells all alike? Note the numerous small cells of nearly uniform size. These are the *somatic cells*, held together by a gelatinous substance. In what part of Volvox are these cells located? Connecting the somatic cells are slender strands of protoplasm. By counting in several instances (not less than six) determine how many of these connecting strands project from each cell. If living Volvox is available, focus on the edge of a specimen, and find *flagella* projecting from each cell. Does the beating of these flagella result in movement of the individual cells, or of the whole organism? From the structure of Volvox, would you say the cells are independent of each other?

Besides the small somatic cells, observe the larger bodies in Volvox. These are either *reproductive cells* or *daughter individuals* derived from them. Determine where they are located. The reproductive cells are of three kinds: (*a*) *parthenogonidia*, which by cell division give rise to daughter individuals asexually; (*b*) *ova* or *eggs;* and (*c*) *spermatozoa* (male reproductive cells).

4*a*. *Parthenogonidia.*—Look for these in very small (young) individuals. They are somewhat larger than the somatic cells and rarely as many as a dozen in number. Some of them may be found to have divided into two, four, eight or more cells forming small *daughter individuals*. In older individuals look for daughters of various sizes. There may be from four to nine of these. Eventually they break out of the parent.

4*b*. *Ova* may be 30 to 100 in number in certain species or as few as four to eight in others. They are considerably larger than the somatic cells. Find ova with *spiny shells* covering them. The shell indicates that they have been "fertilized" by a spermatozoön and have gone into a resting

condition. The fertilized ova later give rise to new Volvox. How many ova in the specimens studied?

4*c*. *Spermatozoa* occur less frequently than ova. When present, they are in bundles like sticks of wood in a rick. Several of these bundles may sometimes be found together. If you do not find them, ask to have them pointed out.

Draw a specimen having parthenogonidia or daughter Volvox, representing the whole organism in outline, and the parthenogonidia or daughter Volvox more in detail. The outline should be at least 3 inches in diameter. Show the somatic cells in a portion of the figure. *Draw* a similar figure of a specimen containing ova and spermatozoa, representing some of the germ cells in detail, and showing somatic cells in part of the figure. May any advance in complexity of Volvox over Pleodorina be observed? If so, in what respects?

5. Hydra is a fresh-water animal found in lakes, ponds, and streams, attached to the surface of dead leaves, aquatic plants, and other objects. Two species are commonly found, the brown hydra (*Hydra oligactis*) and the green hydra (*Hydra viridissima*). Study a living specimen in a salt cellar containing a small amount of water. Examine with the unaided eye, with the dissecting microscope, and with the low power of the compound microscope.

5*a*. *Somatic Cells.*—Focus on the margin of the body, and note a clear outer layer of cells, the *ectoderm*. The darker part within is another layer of cells, the *endoderm*.

Mount a specimen on a slide, supporting the cover-glass so as not to crush it. Focus on the margin. The serrations found there indicate roughly the extent of the principal cells of the ectoderm. Among these, find numerous round bodies smaller than the ectoderm cells, the *nematocysts*, or *stinging organs*. The nematocysts are lodged in cells called *cnidoblasts* which may not be visible in the living animal. In what part of the animal are the nematocysts most abundant? Do you find groups of them anywhere? The structure of the nematocysts should be studied from specimens prepared for this purpose.

Examine mounted cross-sections of Hydra. Note the two layers of cells, the *ectoderm* and the *endoderm*, surrounding the *digestive* or *gastro-vascular cavity*. The bulk of the ectoderm is made up of the cells previously observed as serrations at the surface, approximately rectangular in section and not very deeply stained. These are called *epithelial cells*. Among the epithelial cells are pear-shaped or oval bodies, the *nematocysts*. Look for the cnidoblasts in which the nematocysts are contained. Numerous small deeply stained cells among the bases of the epithelial cells are called *sub-epithelial cells*. From the sub-epithelial cells are derived the cnidoblasts, and some other cells.

Study also the endoderm. Are there distinct types of cells in this

layer, or are all approximately alike? How do the cells of the endoderm differ from those of the ectoderm? What are the large clear spaces in the endoderm cells?

Draw a small portion of a section, showing all the different kinds of cells you have studied. Choose for drawing a portion of a section where the cells are as diagrammatically arranged as is possible to find. The figure should represent the thickness of the two layers as about two inches.

5*b*. *Germ Cells.*—If available examine a living specimen bearing one or more *spermaries* or *testes*. What is the shape of this organ? Do you note any movement within the spermary? The moving bodies are the *spermatozoa*. *Sketch* an entire specimen showing the spermaries. (Use a prepared slide if a live specimen is not at hand.) Examine a cross-section of Hydra through a spermary. The spermatozoa are deeply stained cells in a dense mass. What is their relation to the ectoderm and endoderm?

Examine either a living or a stained specimen bearing an *ovary*. *Sketch* to show this organ. What is the relation of the ovary to the ectoderm and endoderm? (When an ovum is fertilized by a spermatozoön, it develops into an embryo. See demonstration.)

Are the somatic cells of Hydra all alike? If not, how many kinds may be observed? Are the cells of one kind grouped together, or scattered over the body? If the answer to the last question is different for different kinds of cell, specify the difference in your notes. Are the germ cells all alike?

Do you observe any advance in complexity of Hydra over Volvox?

6. The Earthworm (Lumbricus terrestris).—Study both living and preserved specimens.

6*a*. *External Features.*—Note that the body is divided into segments known as *somites* or *metameres*. A segmented animal is said to be *metameric*, or to exhibit *metamerism*.

Observe that the animal has a *dorsal* or upper surface, and a *ventral* or lower surface. It has also an *anterior* end and a *posterior* end. Consequently it has also a *right* and *left* side. Since the earthworm can be divided by only one plane into two corresponding halves, it is said to be *bilaterally symmetrical*. Where does this one plane pass?

The following external features are referred to in the dissection:

Setæ, minute horny bristles arranged in rows on each side of the body. Pass a preserved worm through your fingers in both directions. What does the result indicate?

Clitellum, a swelling of the body in the region of metamere 32. On its ventral side is a pair of thickened ridges, the *tubercula pubertatis*.

Prostomium, a small rounded projection at the anterior end, overhanging the mouth.

Mouth, an opening at the anterior end leading to the *buccal* or *mouth cavity*.

6*b*. *Internal Structure.*—Handle the specimens with care. They must not be cut up or destroyed except as indicated later in the instructions.

Find the *dorsal* side of the animal. Insert the point of the scissors through the body wall a little behind the clitellum near the mid-dorsal line. Be sure that the scissors do not pass into the internal organs. Now cut the body wall backward to the posterior end, then forward to the anterior end, the cut always passing close to the mid-dorsal line. Be especially careful near the anterior end, about the third somite, not to injure the brain.

Separate the cut edges a little, just behind the clitellum, and note the transverse partitions or *septa* (singular *septum*), which divide the *body cavity* or *cœlom* into compartments. The cœlom surrounds the digestive tract. Note the relation between the septa and the intersegmental furrows on the surface of the worm.

Now cut the septa carefully on each side for about an inch. The best instrument for this is the point of a sharp dissecting needle. Lay the worm ventral side down in the dissecting pan and pin the body wall out flat as far as the septa have been cut. Slant the pins outward so as to leave room to work between them. Then with the point of the needle cut or tear the septa forward and backward, putting in pins whenever necessary. When this dissection is completed the septa should have been cut to the same depth on each side. Be careful not to injure any of the internal organs. Remember the general rule in dissection, to cut nothing unless you know what it is and why you cut it.

Readjust the pins in the anterior region so that they pass through the walls of the fifth, tenth, and fifteenth somites. This will facilitate counting them in locating the organs. Now study the following systems of organs.

6*c*. *Reproductive System. Male Organs.*—In somites 9, 11, and 12 notice the three pairs of whitish bodies partly covering the alimentary tract. These bodies are the *seminal vesicles*. In them are located the *testes* which produce the spermatozoa.

None of the remaining male organs are visible without careful dissection. They may be omitted from the study of the dissection but should be studied from a chart and from figures in the text-book ("Principles of Animal Biology," by Shull, La Rue and Ruthven).

Female Organs.—These consist of the paired *ovaries* in the 13th somite and a pair of oviducts in the 14th. Both organs are small and need not be found, but should be studied from a chart or text figures.

Close to the septa separating somite 9 from 10 and 10 from 11, are two pairs of small whitish bodies, the *seminal receptacles*. Mature spermatozoa received from another worm are stored in these. Be careful in the course of the dissection not to remove or injure the reproductive organs.

6*d*. *Blood System.*—The dorsal blood vessel may be seen in the living worm. In favorable specimens it may be seen to pulsate. In the dissected worm it is found imbedded on the dorsal side of the digestive tract. Follow the dorsal vessel forward. In somites 7 to 11 inclusive, will be found certain paired, tube-like red bodies (variously colored in preserved worms), the *hearts*, which are connected with the dorsal blood vessel. The hearts extend ventrad, forming semicircular loops on each side of the digestive tract. They unite below with a *ventral blood vessel*, which extends backward along the ventral side of the digestive tract. If the hearts cannot be seen carefully dissect away the remaining portion of the very prominent septa which obscure the hearts and other organs of somites 7 to 12. The ventral vessel will be seen later in cross-sections. In life the hearts propel the blood from the dorsal to the ventral vessel. Smaller vessels are found throughout the body. Some of the more prominent of these may be found in each segment back of the hearts connecting the dorsal blood vessel with the body wall and the intestine. What is the function of the blood system? How is this function served in Hydra?

6*e*. *Digestive System.*—This consists of a tube extending through the whole length of the body. It is modified into various parts which may be readily found. Beginning at the anterior end these are taken up in order.

The *mouth* has already been found. It leads into the *mouth cavity* or *buccal pouch* in the first three somites. Be careful not to injure the *brain*, a whitish bi-lobed structure situated on the dorsal side of the mouth cavity in somite 3.

The *pharynx* is the thick-walled portion following the buccal pouch. It extends to about the 7th somite. The walls are firm and muscular. Test the consistency of this structure with your dissecting needle.

The *esophagus* is a long slender portion behind the pharynx. It is partly covered by the hearts and reproductive organs, and in the anterior part by heavy septa. The hearts and reproductive organs must not be removed or injured but the reproductive organs may be carefully turned aside in order to reveal the esophagus.

The *crop* is an enlargement following the esophagus. It is situated directly behind the last pair of seminal vesicles in somites 15 and 16 (usually). Feel of it to determine whether it is thick or thin walled.

The crop is followed by the whitish *gizzard*. Feel of this organ to determine whether it is thick or thin walled. Behind the gizzard, the intestine extends to the posterior end of the worm where it opens to the exterior by means of the *anus*.

Compare the digestive system of the earthworm with that of Hydra.

6*f*. *Excretory System.*—Find, with the dissecting microscope if necessary, a pair of coiled tubes in each somite except a few at the anterior and posterior ends. They are located between the septa and partly beneath

the intestine. These tubes are the *nephridia* (singular *nephridium*), or excretory organs. Does Hydra possess any definite excretory organs?

6*g*. *Nervous System.*—In the third somite is a small whitish bi-lobed structure, the *brain*, resting on the buccal pouch. In the posterior part of the worm push the intestine aside, and note the white *nerve cord*. How far does it extend forward and backward? The thickenings of the nerve cord are the *ganglia*. Note the small nerves running out from the ganglia. Find the connection between the brain and the nerve cord in the anterior portion. The connecting cords are called the *circumpharyngeal connectives*.

6*h*. *Muscular System.*—The *longitudinal muscles* are visible in the dissection as glistening strands running lengthwise on the inner surface of the body wall.

Make a *drawing* of the first 25 somites of the dissection three times natural size, putting in all the organs that can be seen in a dorsal view. Turn aside the seminal vesicles of one side so that the underlying organs can be exposed and included in the drawing. Label all the parts identified and shown and indicate somites 1, 5, 10, 15.

6*i*. Examine prepared slides of cross-sections of the earthworm under the dissecting microscope. Observe again the cœlom or body cavity directly between the body wall and the intestine. The intestine is seen in the middle of the section. Determine the dorsal and the ventral sides of the section. This may be done by using some of the following organs as landmarks.

In the intestine note the *typhlosole* which is an infolding of the dorsal wall of the intestine. On the dorsal side of the intestine is the dorsal blood vessel. Beneath the intestine is the *ventral blood vessel*, supported by a thin membrane or *mesentery* seen in the cross-section as a wavy line. Near the ventral blood vessel is the *nerve cord*. In the cœlom may also be found portions of *nephridia* and sometimes portions of *septa*.

The body wall consists of four distinct layers. Lining the cœlom is a very thin layer of cells, the *peritoneum*. Outside of this membrane is a layer of more or less feathery appearing structures, the *longitudinal muscles*. Outside of these is a layer of *circular muscles*. External to these is the *hypodermis*. How many layers of cells in it?

The intestine also consists of four layers. On the inside is a single layer of slender *epithelial cells*. Outside of this is a *circular muscle layer;* then a longitudinal muscle layer reduced to a few fibers; and covering the intestine is a layer of thick *peritoneal* cells.

Draw an outline figure showing the form and position of the various layers of tissue and other organs in outline, but do not fill in details. The boundaries of the layers and organs are sufficient. Be careful to make this drawing with the dorsal side toward the top of the page.

Does the earthworm possess germ cells and somatic cells? If so,

where are the germ cells? Are they of more than one kind? Examine preparations of ovary and of seminal vesicles to secure facts for the answers to these three questions. How many kinds of somatic cells are there? Compare their distribution over the body with their distribution in Hydra. Which arrangement appears to you the more complex? The more specialized? What are organs? Systems? Does Hydra have any approach to organs? If so, where?

B. AGGREGATIONS OF MANY-CELLED INDIVIDUALS

7. Bugula belongs to a group of animals known as Bryozoa. They are found in both fresh and salt water. Bugula is a salt water form. Study a branch in a watch glass. Note the plant-like form. It lives attached to rocks and other objects in the water.

Study the method of branching. In a stained branch on a prepared slide, note how the individuals are arranged.

From a prepared slide examine a favorable individual with a low power of the microscope. Note the transparent *sheath* surrounding the individual, the tentacles surrounding the mouth, and the form of the remainder of the body. Are all individuals alike?

Sketch several individuals including the sheath as seen under the compound microscope.

8. Obelia is an animal related to Hydra (a member of the phylum Cœlenterata). It grows in plant-like colonies on wharves and rocks in salt water. Under the dissecting microscope note the tree-like form of a single branch. Specimens in watch glasses or mounted permanently on slides may be used for this purpose and for the identification of the kinds of individuals and their parts indicated below. Use the compound microscope for parts of this study.

8*a*. *Hydranths* or *zooids*, bearing tentacles, are located at the ends of the branches. Each hydranth is enclosed in a cup-like sheath or *hydrotheca* which is a continuation of the tough membranous covering of the whole colony (the *perisarc*).

In an expanded hydranth note the body with the *hypostome*, an elongated projection in the midst of the tentacles. The fleshy continuation of the hydranth into the stalk is the *cœnosarc*. The cavity in the body of the hydranth continues through the cœnosarc.

8*b*. *Gonangia* (singular *gonangium*), club-shaped individuals usually found in the angles between the hydranths and the main stalk. Note that they have no tentacles, hence can capture no food. How can they be nourished? The sheath forming the outer portion of the gonangium is the *gonotheca*. The fleshy core of the gonangium is the *blastostyle*. Upon the sides of the blastostyle find

8*c*. *Medusæ*, here in an immature form, mere rounded projections. In the larger ones, the beginning of the *tentacles* may be seen at the margin

(see demonstration). The medusæ detach themselves later from the blastostyle, emerge from the gonangium through an opening at the tip which in younger gonangia is plugged up by the broad end of the blastostyle, and live a free swimming existence. Examine one of the free medusæ in a demonstration. Note the *manubrium* in the center. It is homologous with the hypostome and contains the *mouth*. Four *radial canals* extend out from the manubrium as far as the *circular canal* along the margin. The *reproductive organs* are usually borne on the radial canals. The convex side of the medusæ is called the *ex-umbrella* and the concave side the *sub-umbrella*. The medusæ reproduce by eggs and spermatozoa. The structure of a medusa may be better appreciated from an examination of larger medusæ belonging to other species such as Gonionemus or Polyorchis.

The tree-like branch you have examined is not an entire colony. In a demonstration, note that numerous such branches may be connected by a horizontal creeping portion, the *hydrorhiza*, from which the branches arise.

Obelia illustrates a simple form of *polymorphism*, in that it comprises three kinds of individuals in differing form: (1) The hydranth or nutritive individual; (2) The blastostyle which produces (3) Medusæ, the dispersing members of the species.

Draw a branch showing a hydranth and a gonangium in detail. *Draw* also a medusa.

9. Physalia, the "Portuguese Man-of-war," will be on demonstration. It is a very complex polymorphic colony. The various individuals cannot be made out without more careful examination than the demonstration will permit.

C. SUMMARY

What is the simplest form of aggregation into which cells may enter? What animals show this simple collectivism? Are these aggregations colonies, or individuals?

Arrange the animals you have studied in order of ascending complexity. What is the first increase in complexity beyond the simplest condition mentioned above? Point out what are the further increases in complexity, step by step, through the rest of the series.

Is there division of labor in any of these types of aggregation? If so, which ones? Is division of labor lacking in any of them? Is there any coöperation, not involving division of labor, in any of them? If so, where?

Is there any parallelism between aggregations of cells and aggregations of individuals? If so, in what respects?

Do you see any value in the construction of a scale of complexity such as the foregoing? If so, indicate its use.

EXERCISE VI

REPRODUCTION

A. ABIOGENESIS

Living organisms come into existence only from other organisms through some form of reproduction. It was once supposed that living things were sometimes produced directly out of non-living matter, an old theory now referred to as *abiogenesis*.

The following experiment which is to be performed as a class demonstration represents in a simple way the kind of experiment by which abiogenesis was disproved. Preparations for this experiment are to be made as follows: Into each of several clean, sterilized petrie dishes or test-tubes place a small amount of nutrient agar solution; put covers on the petrie dishes and cotton plugs in the test-tubes and sterilize under 15–18 pounds steam pressure. One-half of the preparations are opened in the presence of the class, exposing the agar to the air of the laboratory for about fifteen minutes. The unexposed preparations are kept as controls.

Examine the dishes at frequent intervals for signs of growing organisms (molds, bacteria colonies, and yeasts). On which cultures do they appear first? Source of the growths? Discuss possible sources of contamination in the controls[1] if growths occur in them.

B. TYPES OF REPRODUCTION

Living organisms give rise to other organisms like themselves; that is, they possess the power of reproduction. Since the life of the individual is in every case limited, it is this reproductive capacity that prevents any race from dying out.

Reproduction may be of two general kinds: (1) *asexual* and (2) *sexual*. It is the purpose of this exercise to determine as far as possible, from a limited number of examples, the essential features of each of these types of reproduction.

A. Asexual Reproduction

1. Fission.

1*a*. Fission in a protozoön (Paramecium). Try to find living paramecia that are dividing by means of transverse constriction about the middle, but do not spend much time in search. If living animals under-

[1] Note that air is not entirely excluded by the covers of the petrie dishes. A more careful experiment is not needed, however, to illustrate the method of attacking the theory of abiogenesis.

going fission are found, note the position and depth of the constriction. Look for contractile vacuoles. How many and where? If dividing animals are found watch them at intervals until the process of division is completed.

In specimens stained and mounted on slides, observe carefully the condition of the nuclei. Note that each paramecium has two nuclei, a large *macronucleus* and a minute *micronucleus*. The micronucleus in paramecia which are not undergoing division occurs in or near a little hollow on the side or surface of the macronucleus. Look for it carefully. In fission each of the two nuclei divides, a half going into each of the daughter cells. Each daughter gets one of the old contractile vacuoles and produces a new one. This type of reproduction is called binary fission because each animal divides into two equal parts. Since division is transverse it may also be called transverse fission in contrast to longitudinal fission which occurs in some Protozoa.

Make *two drawings* each two inches long showing an early and a late stage of fission. Represent the body by an outline and make the nuclei dark.

2. Spore Formation.

Spore formation in Monocystis. This is a protozoan parasite found in the seminal vesicles of the earthworm.

Examine a specimen in the cyst stage. The spindle-shaped bodies are the *spores*, contained in the *cyst*. Estimate the number of spores and record it in your notes. All of the spores have been produced by the multiple division of a single cell.

3. Budding.

3*a*. Budding in the metazoön Hydra. Select hydras which bear buds of various sizes, representing stages in the growth of these buds. Note that the cavity of the bud is directly continuous with the cavity of the parent. The bud is formed by the simple outpushing of both layers of cells of the parent's body and the subsequent development of tentacles and mouth. Tentacles are produced by a process similar to budding.

Make an outline *drawing* of parent and bud.

3*b*. Budding in a metazoön, a fresh water sponge. The fresh water sponges exhibit a sort of internal budding. As autumn approaches certain cells in the body wall aggregate into spherical groups and become surrounded by a protecting shell. These spherical bodies are called *gemmules*. Examine a specimen containing them. In some species several gemmules may be enclosed in a common envelope. The adult sponges die in the autumn, but the gemmules live through the winter and develop into new sponges in the spring. Crush a gemmule under a coverglass. Distinguish the whitish cells of the interior from the brownish protective coat. Examine gemmules that have been boiled in *caustic*

soda to destroy the cells inside and to make the gemmules more transparent. You should find a small plain or tube-like *foraminal aperture* through which the small sponge emerges in the spring by an amœboid movement.

Using the compound microscope make a *drawing* of a gemmule an inch in diameter, or show a group of gemmules in a common envelope. Show the foraminal aperture in one of the gemmules.

3c. Budding in a metazoön (Nais, Aeolosoma, Chaetogaster, Dero, or Microstomum). When reproduction by budding occurs the elongated body becomes constricted transversely and later separates into two parts. In some cases the worm may show several budding zones. A demonstration will be provided (living if possible). A sketch in your notes may be helpful, but is not expected on your plates. Be sure to note the name of the worm studied.

How many parents are concerned in each of the observed cases of asexual reproduction?

B. Sexual Reproduction

4. Conjugation in Paramecium.—Look in the cultures for paramecia swimming about in pairs side by side. Such specimens are conjugating. The nuclei of conjugating specimens can be studied only with the aid of prepared slides. The essential part of the process is the exchange of portions of the micronuclei. Several demonstrations of this stage will be provided. *Draw* carefully, representing the body in outline, and the nuclei in detail. Read Chapter VIII in "Principles of Animal Biology," by Shull, La Rue and Ruthven, for an account of conjugation.

5. Reproduction in an Hermaphroditic Metazoön, the Earthworm.—Recall your dissection of the earthworm. Each individual was found to possess both male and female organs. Such an animal is called an *hermaphrodite*. An earthworm does not, however, fertilize its own eggs; each egg is fertilized by a sperm received from another worm and stored in one of the seminal receptacles (spermathecæ). Make a list of the organs which are classed as female and male and be sure that you know the function of each.

Examine a demonstration of ova (female germ cells) in the ovary, and also a demonstration of male germ cells in various stages of development secured from the median seminal vesicles. Read Chapter VIII in "Principles of Animal Biology" for a discussion of reproduction in the earthworm.

How many parents are concerned in sexual reproduction in each of the cases studied? Were the parents alike or unlike?

In a majority of species of animals the parents are unlike in structure, each parent having but a single set (male or female) of reproductive organs. Such species are known as dicœcious while those which have both sets of

sexual organs in the same individual are known as monœcious or hermaphroditic animals. Make a list of at least ten species of animals which are dicœcious. The anatomy of the sexual organs of a dicœcious species will be studied later, in the exercise on breeding habits.

C. Parthenogenesis

The eggs of certain species of rotifers, crustaceans, insects, and others normally develop without fertilization.

6. An Aphid.—A laboratory experiment will be conducted using the aphid or plant louse, Macrosiphum, and the chrysanthemum as a host plant. Several chrysanthemum plants should be carefully examined to discover if they are free from plant lice. If they are free then a single immature plant louse should be placed on each plant, and the plant should be covered with a lantern globe closed at the top with cheese cloth or muslin. The plants will now be placed on a shelf and cared for by an assistant. Make a record of date and just what was done. After a time interval of a number of days count and record the number of individuals on each plant, the date, the number of days elapsed since the lice were put on the plants. If the interval has been long enough some of the progeny may also have borne young.

7. Rotifer.—At the option of the instructor a second experiment to be performed by each student may be instituted. Secure from the instructor or an assistant a Syracuse watch glass with a single immature female rotifer in a small quantity of liquid. Examine under a dissector to make sure that only a single rotifer is present. Now fill the dish two-thirds full of distilled water and food materials as the instructor directs. Write your initials with pencil on the ground edge of the watch glass and put the dish on the shelf designated by the instructor. The assistant will see that dishes are covered and food provided at proper intervals. The record should consist of the date and the number of rotifers put in the culture. After a certain time interval to be announced by the instructor examine your watch glass culture, record the number of rotifers present, date, and interval of time elapsed since the beginning of the experiment.

How many parents were concerned in the act of reproduction in Macrosiphum? In the rotifer? How can you be sure? Why is this classed under sexual and not under asexual reproduction? If in doubt on the latter point ask for a demonstration of a rotifer showing the egg.

C. COMBINATIONS OF ASEXUAL AND SEXUAL REPRODUCTION WITH DIFFERENCES IN STRUCTURE. ALTERNATION OF GENERATIONS OR METAGENESIS

Alternation of generations is a phenomenon exhibited in the life cycle of certain animals in which asexual individuals give rise to sexual indi-

viduals, which in turn produce asexual individuals. The asexual and sexual individuals are *structurally unlike.*

8. Metagenesis in a Colonial Hydroid Obelia.—Recall your drawings of Obelia or if you failed before to work out its structure do so at this time. Note especially:

8*a*. That the hydranths produce gonangia and hydranths by budding.

8*b*. That the gonangia produce medusæ by budding.

8*c*. That the medusæ produce hydranths by means of eggs which must be fertilized by spermatozoa.

8*d*. That the kinds of individuals that reproduce asexually are structurally very unlike the kind which reproduces sexually.

Therefore, Obelia exhibits "Alternation of Generations" or "Metagenesis."

D. SUMMARY

The summary should consist of discussions of abiogenesis, and of asexual and sexual reproduction or a comparison of the two modes of reproduction pointing out distinguishing or essential features of each.

EXERCISE VII

BREEDING HABITS OF VERTEBRATE ANIMALS

A knowledge of the anatomy of the reproductive organs is essential to an understanding of the breeding habits of vertebrates. In order to gain this knowledge the student should work out the structure of the male and female reproductive systems in the frog, using for this purpose dissections which are placed on the table. He should also consult the charts which will show the relative location of the organs and their connections. Examine also a model of the frog showing organs, and specimens partly dissected.

A. ANATOMY

1. Male Reproductive Organs.—In the dissection furnished you note:

1*a*. *The kidneys,* two flattened oval structures side by side. Near their anterior ends find:

1*b*. The *testes* (singular *testis*), two yellowish bodies of ovoid shape. Push one of them aside and observe:

1*c*. The *vasa efferentia* (singular *vas efferens*), delicate white tubes passing between the testis and the median edge of the kidney.

1*d*. The *ureters* are tubes, one passing backward from the lateral margin of each kidney. They connect the kidneys with:

1*e*. The *cloaca,* a short passage which is a continuation of the large intestine. (The large intestine and part of the small intestine are included in your specimen.) The cloaca discharges to the exterior through the anal aperture. If your demonstration specimen is from the species *Rana pipiens,* find also:

1*f*. The *Muellerian ducts,* two irregular white tubes extending from the cloaca forward to a point in front of the kidneys. They correspond to the oviducts of the female, but are functionless in the male.

Make a *diagram* of the male reproductive system. Discover if possible how the spermatozoa reach the water.

2. Female Reproductive Organs.—In the demonstration dissection furnished find:

2*a*. The *ovaries,* two large lobed masses containing black and white eggs (or the ovaries may be much smaller and white).

2*b*. The *oviducts,* two thick convoluted tubes extending longitudinally beside the ovaries.

2*c*. The *uterus,* a thin-walled portion of the posterior end of each oviduct. Each uterus connects with:

2*d*. The *cloaca*, a continuation of the large intestine, as in the male. Try to discover how the eggs escape into the water.

Make a *diagram* of the female reproductive system. Indicate the path of the eggs by means of arrows.

Compare your diagrams of the reproductive systems of the frog with the charts showing similar diagrams for the other vertebrates. Be sure that you understand the function of each organ in the frog and in a mammal.

B. METHODS OF REPRODUCTION AND THE TYPES OF EGGS

Full notes on this exercise are desired and particular attention must be given to the conclusions or summaries called for under paragraphs 3*c*, 4*d*, and *E*.

3. Types of Eggs of Oviparous Forms.

3*a*. Examine the eggs of two fishes (perch and white-fish) and three amphibians (a frog, a toad and a salamander *Ambystoma tigrinum*) which are deposited in water and fertilized as laid. Describe the covering and the differences in the way in which the eggs are held in a mass.

3*b*. Examine the egg of a turtle, a crocodilian, a snake and a bird which in each case is fertilized within the body of the mother and subsequently laid in places exposed to air. Describe the difference in the texture of the shell of the two types 3*a* and 3*b*. Read the paragraph on shell structure in the text-book.[1]

3*c*. Read the account of fertilization given in the text-book and explain the relation between the habits of the animals in 3*a* and 3*b* and the nature of the egg-covering.

4. Types of Eggs of Animals Which Give Birth to Young (Ovoviviparous and Viviparous Forms).

4*a*. Examine the demonstrations of the developing eggs in position in the body of an ovoviviparous reptile (the garter snake). Describe the position of the eggs in the genital system and their relation to the body of the mother.

4*b*. On slides prepared for the purpose locate the eggs in the ovary of a viviparous species (the cat, for example). Note the relative size of the eggs.

4*c*. Examine the demonstrations of a mammalian embryo (mouse) in position in the uterus and describe the relation of the developing young to the body of the mother.

4*d*. Give an explanation of the differences in the relative size of the egg in oviparous, ovoviviparous and viviparous forms.

[1] The frequent references to the text-book in this section apply to "Principles of Animal Biology," by Shull, LaRue and Ruthven, Chapter IX.

C. BROODING HABITS

5. The Habit, Among Oviparous Forms, of Guarding the Eggs Without Incubating Them.

5*a*. Read the section in the text-book describing this habit.

5*b*. Examine the demonstration specimens, or in the absence of these the figures of the marsupial frog (Nototrema) and a fish which carries the young (Hippocampus), noting the position of the brood pouch.

6. The Habit of Brooding the Eggs (Incubation) and the Habit of Brooding the Young.

6*a*. Read the section in the text-book describing these habits. Examine the series of bird and mammal nests in the laboratory, and describe at least three nests representing different types of construction.

6*b*. Note the position of the brood pouch in a marsupial (opossum), or in the figures of a kangaroo in the text-book.

D. BIRTH STAGES

7. Oviparous and Ovoviviparous Species with a Larval Period.

Compare the young and adult of the common lamprey, a frog and the salamander *Ambystoma tigrinum*. Describe the differences in the mouth, eyes, form of body and appendages.

8. Species Without a Larval Period.

Compare the newly born young of a shark, a garter snake, two birds (English sparrow and the chick), a mouse and a guinea pig. Describe the differences in the stage of development at time of birth as shown by the relative size, strength, the covering of scales, hair or feathers, and the eyes.

E. SUMMARY

Do you discern any possible relation between the oviparity, ovoviviparity or viviparity of an animal and the number of eggs it produces? Any relation of the same three phenomena to the certainty that the eggs will be fertilized? Any relation to the mode of life of the animal? Discuss these relations if they appear to exist.

EXERCISE VIII

EMBRYOLOGY OF TYPICAL ANIMALS

A. MATURATION AND FERTILIZATION

The change undergone by the male and female germ cells previous to fertilization is known as *maturation.*

1. Maturation of the Male Germ Cells.—The undifferentiated male germ cells are known as *spermatogonia.* These multiply by ordinary mitosis. When mitosis stops each cell increases in size and is known as a *primary spermatocyte.* Each primary spermatocyte divides into two *secondary spermatocytes.* Each of these in turn divides into two *spermatids* which metamorphose into *spermatozoa.* Thus out of each primary spermatocyte four spermatozoa are formed. During the process of maturation the number of chromosomes is reduced one-half. A wall chart should be studied for the outline of the process.

1*a*. Examine sections of the testis of the beach grasshopper, *Trimerotropis maritima.* At one end of each section spermatogonia will probably be found, at the other end mature spermatozoa, and between the two ends spermatocytes in various stages.

1*b*. Note the small size of the spermatogonia. Find some undergoing mitosis. In a polar view of an equatorial plate determine as nearly as possible the number of chromosomes. Record the number.

1*c*. The spermatocytes are larger than the spermatogonia. Among them find cells undergoing mitosis. From an anaphase of the division of a secondary spermatocyte determine as nearly as possible the number of chromosomes. If in doubt whether you are observing the correct stage ask to have one shown to you. How does the number of chromosomes in each anaphase group in the secondary spermatocyte compare with the number in the spermatogonia? The number in these anaphase groups is the number that goes into the spermatozoa.

1*d*. *Draw* a group of mature spermatozoa, either from the grasshopper or from a mammal, of which a demonstration may be provided.

2. Maturation and Fertilization of Female Germ Cells.—Since, in the animal selected for the study of the female germ cells, the processes of maturation and fertilization occur in large part simultaneously, they are studied together. The chronological order of events is followed.

2*a*. Examine sections of the uterus of *Ascaris megalocephala.* See a specimen of Ascaris. The large rounded bodies are *oöcytes* or later stages.

The nature of these sections has been explained in Exercise IV. While still in the ovary, before growth began, the female cells were *oögonia.*

2*b.* In the uppermost row of sections which is from the inner part of the uterus, find *primary oöcytes* each containing a triangular dark body with a distinct black nucleus. These triangular bodies are *spermatozoa* which have already penetrated the primary oöcytes. Some spermatozoa may also be found among the oöcytes.

2*c.* Note the nuclei of the primary oöcytes. Some will have formed spindles preparatory to the first maturation division.

2*d.* In the second row of sections, from a point a little lower down in the uterus, observe oöcytes undergoing their first maturation division. The chromosomes are arranged in two quadruple bodies or *tetrads.* Each tetrad is composed of two chromosomes brought together in a process known as *synapsis,* the chromosomes of the pair having divided so as to form four parts. The nucleus of the spermatozoön, surrounded by more darkly stained protoplasm, may also be seen in some specimens. Select a clear specimen, and *draw.*

2*e.* In the third row of sections are *secondary oöcytes* undergoing the second maturation division. The secondary oöcyte is surrounded by a thick membrane. Within this membrane, at the surface of the oöcyte, is found in some sections, a small dark object, the *first polar body.* This and the secondary oöcyte constitute the two daughter cells formed by the first maturation division described in 2*d.* If the polar body is not seen, explain its absence.

In the secondary oöcyte observe the spindle, bearing two double bodies, the *dyads.* Each dyad is half of one of the tetrads described in 2*d.* When this second division is completed, two single bodies (*chromosomes*) will have gone into the *second polar body* (a very small cell), and two remain in the *mature ovum.* The nucleus of the spermatozoön may be visible in some specimens.

Draw a specimen showing a spindle with clear dyads.

2*f.* In the fourth row of sections, the second maturation division is already completed and the two polar bodies are visible at the surface of the *mature egg* in some of the specimens. (The first polar body in some instances adheres to the inner surface of the egg membrane.)

The first polar body in some animals divides so that out of the original oöcyte, four cells are formed, one of which is the mature egg and the others the polar bodies which are without function.

Observe in the interior of the mature egg the two large vesicular nuclei, containing scattered granules of chromatin. One of these is the *egg nucleus,* the other the *sperm nucleus.* These fuse to form a *cleavage nucleus* and this fusion constitutes the final step in *fertilization.*

2*g.* In the fifth row of sections, the fertilized ovum is undergoing division or cleavage. Two-celled and four-celled *embryos* will be found.

In a favorable specimen count the chromosomes in one cell. This number is the number of chromosomes found in all the body cells of Ascaris, and is known as the *somatic* or *diploid* number. Recall the number of chromosomes in the mature ovum (see 2*e* above), which is known as the *reduced* or *haploid* number. The diploid number is restored at fertilization.

Compare maturation in the male and female germ cells (see chart).

B. DEVELOPMENT

Development of the Frog

The developmental processes of the various groups of vetebrates are quite similar. The development of one of them, therefore, serves to illustrate the process in all, just as the formation of spermatozoa and ova in the grasshopper and Ascaris are typical of the corresponding processes in other animals.

Study the early development of the frog, using the following stages:

1. During the First Day.

1*a*. Unsegmented Egg. Study with dissecting microscope, using transmitted light and reflected light (the latter preferably with dark background). The middle of the black half is called the *animal pole;* the middle of the white half the *vegetative pole.* Note the layers of *jelly.* How many? Relative thickness? *Draw* an unsegmented egg, side view, with the animal pole toward the top of the plate. Make the egg itself ¾ inch in diameter and the jelly in proportion. Label.

1*b*. Two cell stage. Note the *cleavage furrow.* Where is it deepest? *Draw* the egg without the jelly, side view, and with the animal pole above. Label the poles and furrow.

1*c*. Four cell stage. A second cleavage furrow is present. How is it placed with respect to the first? *Draw,* without the jelly, inclining the animal pole slightly toward you so as to show the intersection of the two cleavage furrows.

1*d*. Either twelve cell or sixteen cell stage. The cells of the animal half of the egg divide more rapidly than those of the vegetative half, so that there may be eight cells in the region of the animal pole while there are only four near the vegetative pole. This would be a twelve cell stage. The four vegetative cells soon divide, making sixteen in all.

Note that the eight cells in the animal half are usually arranged roughly in two rows of four cells each. Compare these cells in size with those about the vegetative pole. *Draw* the twelve cell or sixteen cell stage, inclining the animal pole toward you so as to show the entire group of eight cells above, but still representing an oblique side view. Label the animal and vegetative poles.

2. During the Second Day After Laying.

2*e*. Early *gastrula*. Previous to this stage the cells have through successive division become small and numerous, the whole mass forming a hollow ball known as the *blastula*. During the early gastrula stage the cells near the border between the black and white areas begin to be tucked into the hollow interior of the mass, along a crescent-shaped line. This crescentic opening is the *blastopore*. Note that the cells on one side of it are white, on the other side black. *Draw*, with the blastopore in the middle of the figure, convex side up. Label animal and vegetative poles.

2*f*. Late gastrula. The invagination of the cells into the interior is now occurring along a circular line, that is, the blastopore is now a circle. The white cells within this circle constitute the *yolk plug*. The yolk plug is all that is left of the vegetative half of the egg that has not retreated into the interior. A *neural groove* may be present, but will probably not be found at this stage. *Draw*, turning the blastopore nearly to the right side. Label animal and vegetative poles.

2*g*. Neural groove stage. The *neural fold* is a ridge or elevation on the surface of the embryo. The fold is continuous and in the form of an elongated ring, wide at one end and narrow at the other. Later the wide part forms the *brain* and the narrow part the *spinal cord*. The groove between the neural folds is the *neural groove*. At a somewhat later stage the neural folds of the two sides come together above the neural groove and fuse, forming a *neural tube*, which differentiates into the brain and spinal cord. *Draw*, with the dorsal side toward you, that is showing the whole nervous system.

3. About the Fifth Day After Laying.

3*h*. Early *larva*. Note the prominent *tail;* the V-shaped *sucker* under the *head;* and the rounded body, its form due to the yolk still present. At each side of the neck there may be a prominence, the *gill plate*. *Draw*, in side view, but tilt the ventral side up enough to show the sucker. Omit shading.

4. About the Eighth Day, or the Time of Hatching from the Jelly.

4*i*. *Tadpole*. Note the *external gills* developed from the gill plate of an earlier stage; the *operculum*, a fold of skin partially covering the gills and extending entirely across the ventral side of the body; the broad *tail* with its thin margin or *fin;* the V-shaped *segments* or *myotomes* into which the muscles in the axis of the tail are divided; the angular *mouth* beneath the head; the two *suckers* formed by the division of the original one sucker; and the *eye*, a whitish spot surrounded by a darker ring on each side of the head above. The *nasal pits*, minute depressions at the anterior end of the head, will be visible in clean specimens.

Draw a tadpole, tilting up the ventral side enough to show the mouth and suckers. Omit shading.

5. After Several Months or a Year (According to the Species Used).

5*j*. Tadpole with hind legs. Note the following external features observed in the eighth day tadpole: tail, fin, myotomes, mouth, eyes, and nasal pits or nostrils. In addition find:

The *hind legs*. The forelegs are present but concealed.

The *spiracle*, an opening on the left side. Its front edge is the edge of the operculum (see stage 4) which has fused with the body everywhere except at this point. Water passes out of the gill chamber through the spiracle.

The horny *jaws* with which the tadpole scrapes off little particles of food from objects in the water.

The *myotomes* or muscle segments along the sides of the body and tail. Strip off the skin from a part of the tail and with a sharp needle tease apart the muscle fibers of a myotome. Note whether the muscle fibers extend beyond the connective tissue septa.

Internal Features.—Slit open the body wall on the ventral side and turn the flaps back. Observe:

The much coiled *intestine*, and the *mesenteries* supporting its coils.

The *liver*, a brownish body to the right of the intestine (the observer's left), and the *gall bladder*, on the posterior side of the liver.

The *pancreas*, along the anterior part of the intestine.

The *heart* with its whitish *ventricle* anterior to the intestine. Push the intestine to one side and beneath it find:

The *fat bodies*, branching yellow organs.

The two *kidneys* lying against the dorsal wall in the posterior part of the body cavity.

The small *reproductive organs* lying near the anterior ends of the kidneys. It is difficult to distinguish the sexes at this stage.

The *lungs*, two flattened black or grayish structures attached at the anterior end of the body cavity and free at their posterior ends. They are rudimentary and still functionless at this stage.

Open the gill chamber by slitting through the operculum and observe:

The brownish fluffy *gills*. Probe between them into the mouth. The openings from the gill chamber into the mouth and pharynx are the *gill slits*.

The *fore legs* inside of the opercular cavity behind the gills.

C. SUMMARY

Describe carefully, but without too much detail, the essential features of (*a*) maturation and (*b*) development. Treat both phenomena as continuous processes, not as a series of stages. That is, fill in the gaps between the stages studied in the laboratory.

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EXERCISE IX

HOMOLOGY

Structures or organs having a similar embryonic origin irrespective of their final form or their function are said to be homologous. All such structures are believed to have been derived from some common ancestral, more generalized type of structure and to have diverged in various directions. The generalization that the ancestral history of a structure is repeated during embryonic development is known as the *Biogenetic Law* or the *Recapitulation Theory*. It is one of the arguments in favor of the theory of organic evolution.

A. EMBRYONIC ORIGIN OF VERTEBRATE LIMBS

1. Limb Buds of a Toad or a Frog Tadpole.

1*a*. Examine tadpoles showing a very early stage in the development of the hind legs. Note the rounded prominences, the hind limb buds, at the base of the tail. *Draw* an outline of the whole animal, two inches long, in side view, and make the limb buds dark.

1*b*. In an older embryo observe that each limb bud is now elongated, and that the distal end is broadened and shows signs of division into several *digits*. How many? *Draw* the limb bud, considerably enlarged, without the body.

1*c*. Determine the number of digits in the hind foot of an adult frog.

2. Limb Buds of Chick Embryos.

2*a*. Examine a chick embryo after 72 to 80 hours of incubation. Note that the body is in the form of a letter J (see wall chart). The shorter and thicker limb of the J is the *head*, which bears the *eyes*, large rounded prominences on each side. The longer and more slender limb of the J is the *trunk*, the bend of the J being the *neck*.

The *limb buds* are two semicircular prominences on each side of the trunk. Are they alike? *Draw* an outline of the body two inches long, and represent the limb buds dark.

2*b*. Examine a chick embryo after 100 to 120 hours of incubation. The general features may be recognized from the description above. In addition observe that the limb buds are elongated, that their distal ends are flattened, and that the division into digits has begun. (The latter feature is best observed if the light falls obliquely on the flattened surface of the limbs, so as to throw shadows in the hollows.)

Draw the limb, either wing or leg, omitting the body. Compare the origin of the wings and legs of the chick with the origin of the hind legs of the tadpole.

B. DIVERGENCE OF ADULT VERTEBRATE LIMBS

3. Hypothetical Pentadactyl (Five-toed or Five-fingered) Limbs.

Before comparing the adult limbs of the frog, pigeon, and man, study a chart representing the skeleton of a hypothetical pentadactyl limb. The limbs of vertebrates have diverged in various ways from this typical form.

3*a*. *Fore Limb.*—Note the following parts: The *shoulder girdle,* composed of *clavicle, scapula,* and *coracoid,* with perhaps a *precoracoid;* the upper arm or *humerus;* the fore arm composed of *radius* and *ulna;* the wrist with its ten *carpal bones;* the body of the hand with its five *metacarpals;* and the *digits* or fingers composed of *phalanges.* How many phalanges in each?

3*b*. *Hind Limbs.*—Note the following parts: The *pelvic girdle* composed of *ilium, ischium,* and *pubis;* the leg bone or *femur;* the lower leg with its *tibia* and *fibula;* the *tarsals,* ten in number, in the ankle; the five *metatarsals* forming the body of the foot; the digits or toes, composed of *phalanges.* How many in each toe?

4. The Limbs of Man.

4*a*. *The Arm.*—Compare the human arm bones with those of the typical pentadactyl fore limb. Study the following structures: The *pectoral* or shoulder girdle, composed of the *scapula* or shoulder blade, the *clavicle* extending from the shoulder to the sternum or breastbone, and the *coracoid,* a hook-like process fused to the head of the scapula but which in youth starts as a separate center of ossification; the arm bone or *humerus;* the fore arm with its *radius* (on the thumb side) and the *ulna;* the *carpals* in the wrist (number?); the *metacarpals* in the body of the hand; and the *phalanges.* How many in each digit?

4*b*. *The Leg.*—Compare the human leg bones with those of the typical pentadactyl hind limb. Note similarities and differences.

The pelvic girdle is fused into a single bone, the *innominate,* on each side. The *ilium* is the broad expanded portion above the hip socket or *acetabulum.* The *ischium* projects downward and somewhat backward from the acetabulum. The two *pubes* of the opposite sides meet in the middle line in front, from which point two branches project, one upward and outward to the acetabulum, the other backward and downward to the lower end of the ischium.

In the leg proper observe: the *femur* or thigh bone; the *tibia* (larger) and the *fibula* in the lower leg; the *tarsals* in the ankle (number?); the *metatarsals* in the body of the foot; and the *phalanges.* How many in each digit?

5. The Limbs of a Frog.

5*a*. *The Fore Limb.*—Omit the pectoral girdle. Compare the bones of the arm with those of the typical pentadactyl arm. Note the *humerus* in the upper arm, and the *radio-ulna* in the fore arm. Which of the fused bones is the radius? Study also the irregular *carpal* bones of the wrist (number?); the *metacarpals* in the body of the hand, the one of the thumb being rudimentary; the *phalanges*, present in the second, third, fourth, and fifth digits, but wanting in the first. How many in each digit?

5*b*. *The Hind Limb.*—Compare with the typical pentadactyl hind limb. The pelvic girdle consists, on each side, of a long bone the *ilium*, extending forward from the acetabulum; the *ischium*, a rounded flat bone behind the acetabulum; and the *pubis*, a triangular bone below the acetabulum. The latter is more or less translucent in fresh preparations. Each bone forms part of the acetabulum. They may be readily distinguished in the skeleton of a young frog. Observe the *femur* in the thigh, and the *tibio-fibula* in the lower leg. Which edge of the latter represents the tibia? There are four *tarsals*. Two of them are much elongated; beyond these are the other two, small irregular bones. Study also the *metatarsals* in the body of the foot, and the *phalanges* in the toes. How many in each toe? A rudimentary sixth toe may be present on the inner side of the foot.

6. The Limbs of a Pigeon.

6*a*. *The Fore Limb or Wing.*—Compare the bones of the pigeon wing with those of the typical pentadactyl fore limb as well as with the other forms already studied.

The *pectoral girdle* consists of the *scapula*, a sword-shaped bone projecting back over the ribs; a *coracoid*, sloping downward and backward and joining with the sternum or breastbone; and the two clavicles fused to form the *furcula* or wishbone. Observe the *humerus* in the upper arm and the *radius* and *ulna* (larger) in the fore arm. Only two free *carpals* are present and they may be hidden in the ligaments of the wrist. See a thoroughly cleaned skeleton to find them. The remaining carpals are fused with three metacarpals to form a large irregular bone, the *carpo-metacarpus*, consisting of two rods joined at the ends. The larger of the two rods represents the second metacarpal. At its base, on the anterior edge, is a tubercle which represents the first metacarpal. The third metacarpal is represented by the more slender one of the two rods.

Only three digits are represented by *phalanges*. The first finger has a single spine-like or triangular phalanx. The second has two phalanges; and the third finger one, which may be closely applied to the first phalanx of the second finger.

6*b*. *The Hind Limb.*—Compare the leg of the pigeon with the typical pentadactyl limb and with those of the other forms studied.

The pelvic girdle is fused into a single bone except in young birds. The *ilium* is the broad flat part above. The pubis is the slender curved rod at the lower margin of the girdle, behind the acetabulum. It is partially separated from the rest of the girdle by a long cleft. The *ischium* lies above this cleft and below the large opening behind the acetabulum. Observe the *femur* in the thigh, and the *tibio-tarsus* in the lower leg. The *fibula* is a slender bone fused to the tibio-tarsus near its upper end. The *tarsals* are not present as distinct bones, some of them being fused with the tibia and some with the metatarsals. The latter together with the fused metatarsals form the *tarso-metatarsus* of the foot. Note that it is a triple bone, the second, third, and fourth metatarsals being fused. At the proximal end they are fused with some of the tarsal bones. The first metatarsal is a separate bone applied to the inner edge of the tarso-metatarsus at its distal end. There are four digits. The distal *phalanx* of each is modified for the support of the claw. The first digit points backward, the second, third, and fourth forward. How many phalanges in each digit?

Draw two of the limb skeletons studied and label fully.

Prepare a chart comparing the limbs of man, the frog, and the pigeon. In the first column place the names of all the bones found in the limb skeleton of any of the animals studied; in three other columns state whether the bone in question is in any striking way modified from the hypothetical ancestral condition (3*a*, 3*b*), such as fusion with another bone, reduction in number, etc., in the three animals named.[1] If not so modified, leave the corresponding place in the chart blank. What does this chart, when completed, show?

C. MODIFICATION OF LIMBS IN EVOLUTION

Animals possessing homologous structures, no matter how different those structures are in the adult, are believed to be related to one another. That is, they are believed to have descended from a common ancestor at some more or less remote time. If this belief is well founded, these structures must have become modified from the ancestral condition.

While it is easy to demonstrate, as has been done above, the similarity of origin of such homologous structures, it is usually impossible to trace the evolutionary changes by which the similar structures became different. These changes can be certainly known only from the fossils of animals from various points in the line of descent from the common ancestor, and such fossils are usually wanting. For this reason the stages of modification in the frog and bird are not thoroughly known. In the horse, how-

[1] The student should have acquired in this exercise the ability to name most of the bones found in the limbs of any vertebrate. At the option of the instructor, an additional form, such as the horse, may be introduced to test this ability.

ever, a fairly complete series of fossils demonstrates the transition from the many-toed ancestor to the one-toed modern horse. This series of fossils should be examined at the present stage of the work but a careful study of them will be deferred until the exercise in paleontology.

D. SUMMARY

What is homology? What evidence of homology in the foregoing study? Must adult structures be different from one another in order to exhibit homology? Must embryonic structures be alike in order to exhibit homology? Are the wings of two robins homologous with each other? In what ways do the vertebrate limbs differ among themselves? What is the commonest kind of modification from the supposed ancestral type? Which of the limbs studied is the most modified from the ancentral type? Which least modified? On the basis of the structure of the limbs, does man stand high or low in the animal series? Of what use is homology in other branches of zoology?

The above questions are a guide only to the contents of the summary not to the *order* of presentation.

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EXERCISE X

TAXONOMY

Taxonomy (Gr. taxis = arrangement + nomos = a law) is the arrangement of known facts according to law. As applied to animals, taxonomy has for its object the discovery of the pedigree of every animal from an evolutionary standpoint; that is, its kinship or blood relationship, and consequently its position in the animal series or genealogical tree. The characters used in determining such relationship are the form and structure of the adults, young and embryos, since these most nearly indicate the degree of kinship among organisms. The discovery of homologous structures in two or more animals is regarded as a certain indication of kinship.

In the work on taxonomy, numerous sketches should be made, but it is left to the student to decide what forms shall be drawn. As aids to memory, these sketches should serve two purposes. First, they should recall those features of animals which place the animals in certain groups; these features are listed in the following exercises under the heading "Characteristics." Second, many animals should be simply *remembered* as belonging to certain groups, without the necessity of recalling the characteristics which put them in those groups. For this second purpose, the drawings of one phylum or of one class, should be grouped together on consecutive pages.

Drawings are to be made on note paper, not on the drawing sheets.

At the end of the exercise on Taxonomy, the student should be able to place any animal studied in its proper group. Be prepared for a test of your ability to do so.

A. THE PHYLA OF ANIMALS

All animals have been arranged according to their supposed relationships into phyla (Gr. phulon = tribe, race, stock). All members of a single phylum possess certain characteristics in common, and differ in certain of these respects from the members of every other phylum. The principal characteristics of each phylum are listed with illustrative examples, in the following exercises.

1. Phylum PROTOZOA (Gr. protos = first + zoön = animal).

Characteristics.

1*a*. Unicellular. Examine stained specimens of Amœba. The single nucleus, together with the absence of cell boundaries within the animal

demonstrates that it is a protozoön. Living specimens of other protozoa may be stained with acetic methyl green, if desired, to show the existence of but one cell in each.

1*b*. If cells are attached to one another, all are alike. Examine stained preparations of Epistylis and Carchesium.

2. Phylum PORIFERA (Lat. porus = pore + ferre = to bear).

Characteristics.

2*a*. Aquatic, mostly marine. Spongilla is a fresh-water form.

2*b*. Usually radially symmetrical. Examine Grantia or other sponge. How many planes can be passed through the longitudinal axis, each dividing the body into two parts that are mirrored images of each other?

2*c*. Multicellular. Examine a cross-section of Grantia. Note that many cells are present.

2*d*. Diploblastic. Note also in the cross-section of the body that there are two layers of cells, with a gelatinous substance between.

2*e*. Numerous pores. Examine the surface of a dried specimen of Grantia with a dissecting microscope. Also the inner surface of a specimen split open (keep in alcohol or water).

2*f*. Skeleton composed of spicules or spongin. For spicules examine the surface of Grantia, the skeleton of Euplectella, and a slide bearing isolated spicules of Grantia. For spongin, tear off a minute portion of a bath sponge, place between two slides, and examine with a compound microscope.

3. Phylum CŒLENTERATA (Gr. koilos = hollow + enteron = intestine).

Characteristics.

3*a*. Aquatic, mostly marine. Hydra, and at least one of the colonial hydroids, are fresh-water forms.

3*b*. Diploblastic. Examine a cross-section of Hydra. Note the two layers of cells.

3*c*. Radially symmetrical. How many planes can be passed through the longitudinal axis of Hydra, each dividing the body into halves approximately mirrored images of one another? How many such planes through the jelly-fish Gonionemus? Through a hydranth of Obelia? The sea-anemone Metridium? The coral Fungia?

3*d*. Single gastrovascular cavity. Note the hollow interior of Hydra as shown in cross-sections. Observe also the cœnosarc of Obelia.

3*e*. Nematocysts. Examine these in preparations of Hydra and hydranths of Obelia. In the tentacles of Gonionemus. If living material is available, examine nematocysts of Hydra that have been discharged.

4. Phylum PLATYHELMINTHES (Gr. platus = broad + helmins = an intestinal worm).

A. Characteristics

4*a*. May be (1) free-living in water or earth, or (2) parasitic in or on other animals.

4*b*. Bilaterally symmetrical. Examine Planaria. How many planes may be passed through the center of the body, each dividing it into two parts that are mirrored images of one another?

4*c*. Triploblastic. Examine sections. Note a middle tissue between ectoderm and entoderm.

4*d*. Single gastrovascular cavity (may be wanting in parasitic forms). Examine an entire planaria; note the gastrovascular cavity and its branching form. It has but one opening, the mouth, as in the Cœlenterata.

4*e*. Unsegmented. In Planaria, note that the body is not divided into a series of segments.

B. Special Features.

In the tapeworm observe:

4*f*. That the animal is not segmented, but is colonial, the members of the colony being attached in a linear series. Each individual is called a *proglottis*.

4*g*. The *head* or *scolex*. The individuals of the colony are successively budded off from the scolex. Note the *hooks:* also the *suckers*. What is the use of these structures?

4*h*. The absence of a gastrovascular cavity. Why is it not necessary?

4*i*. The reproductive bodies make up the greater part of the body.

5. Phylum NEMATHELMINTHES (Gr. nema = thread + helmins = an intestinal worm). One of the richest phyla in number of species, yet seldom attracting general attention.

A. Characteristics.

5*a*. Cylindrical in form. See Ascaris or any other species available.

5*b*. Bilaterally symmetrical. Meaning of this expression? Verify in Ascaris or any other species.

5*c*. Triploblastic. Meaning of this term? Transparent living forms can usually be had either in mother of vinegar (vinegar "eels") or in old protozoan cultures. If the nematodes keep quiet enough, observe the triploblastic feature. Or examine a cross-section.

5*d*. Unsegmented. See any nematode, *e.g.*, Ascaris.

5*e*. Alimentary canal with both mouth and anus. Demonstrations may be available.

5*f*. Cœlom present. This is a cavity surrounding the alimentary canal. Examine a dissection of Ascaris, and observe that the body wall

may be cut through without opening the digestive tract. Was such a cavity present in any of the preceding phyla?

B. Economic Representatives.

Some of the most dangerous parasites of man and other animals as well as of plants are found among the Nemathelminthes. Among the demonstrations are:

5*g*. Ascaris, parasitic in the intestine of pigs, horses, and man.

5*h*. Trichinella, which causes trichinosis in pigs, rats, and man.

5*i*. Necator, the hookworm, that causes a form of anemia in human beings.

6. Phylum ECHINODERMATA (Gr. echinos = a sea-hedgehog + derma = skin).

Characteristics.

6*a*. All marine.

6*b*. Radially symmetrical. Meaning of this expression? Verify in a starfish; in a sea-urchin. (There are exceptions to radial symmetry, especially in minor features.)

6*c*. Parts usually arranged in fives. Verify in starfish; in sea-urchin; in brittle-star.

6*d*. Generally covered with spiny exo-skeleton of calcareous matter. Observe in starfish; in sea-urchin; in brittle-star. Compare, however, with sea-cucumber.

6*e*. Possess tube-feet for locomotion. These are connected with a water vascular system in the body, and operate by means of suction.

7. Phylum ANNELIDA (Lat. annellus = a little ring.)

A. Characteristics.

7*a*. Mode of life is terrestrial (earthworm), or aquatic (Aeolosoma, fresh water; the sandworm Nereis, marine), or parasitic (some leeches).

7*b*. Segmented. Recall earthworm. Note also the sandworm Nereis. Compare in this respect with the Platyhelminthes and Nemathelminthes, which are also called "worms."

7*c*. Setæ. Re-examine the earthworm. How are the setæ arranged? Observe the sandworm, with the flattened projections at the sides of the body, upon which are bunches of setæ.

B. Special Feature.

7*d*. Suckers in leeches. These attach the animals to the body of the host whose blood they suck. Examine any leeches available.

8. Phylum MOLLUSCA (Lat. mollis = soft).

Characteristics.

8*a*. Body soft and unsegmented. Observe the razor-shell clam Solen, Lampsilis, and Modiola, in which the shell is either open or partly removed.

8*b*. Body bilaterally symmetrical (see Chiton, Anodonta, and the cuttle-fish Sepia), though often apparently asymmetrical (the common snail Polygyra, Natica, or any other snail).

8*c*. Locomotion usually by a fleshy, muscular foot. In Modiola, Lampsilis, and the clams generally, the foot is wedge-shaped. In Polygyra and other snails, it is flat and used for creeping along surfaces. In Loligo, Sepia, and Nautilus, the foot is composed in part of a series of arm-like projections.

8*d*. Body usually protected by a calcareous shell which may consist of two valves (Lampsilis, Pecten, Pinna), of a spirally wound tube (Helix, Polygyra, Nautilus), or be concealed by the fleshy parts (Loligo, Sepia), or wanting (Octopus).

8*e*. Possess a mantle, a thin membranous sheet that secretes the shell. This may be a single piece (Polygyra, Loligo), in two flaps lining the two valves of the shell (Lampsilis, Modiola), or wanting (the slug Limax).

9. Phylum ARTHROPODA (Gr. arthros = joint + pous = foot). The Arthropoda include hundreds of thousands of species, probably a greater number than any other phylum.

A. Characteristics.

9*a*. Segmented. Examine any insect; a crayfish; a centipede; a spider.

9*b*. Paired jointed appendages (legs, antennæ, mouth parts, etc.). How many pairs of legs in an insect? In a crayfish? In a centipede? In a spider?

9*c*. An exo-skeleton of chitin covering the body. Observe in all the forms mentioned in the preceding paragraphs.

B. Special Considerations.

9*d*. Although each arthropod has a definite number of segments in its body, these segments are often fused in characteristic ways so that the number is not easy to determine. The number of appendages, or the embryonic development, is relied on in such cases to establish the correct number.

In insects, a number of segments are fused to form a *head*, others are fused to form a *thorax*, while the segments of the abdomen remain more or less movable upon one another. Make out these regions in Polistes (wasp) and Dissosteira (grasshopper), or other insects.

In the crayfish, the segments of both head and thorax are fused into one immovable group called the *cephalo-thorax*, while those of the abdomen are movable. Make out these regions in Cambarus and Palinurus, or other crayfishes.

In spiders, the cephalo-thorax is one group of fused segments and the

abdomen is also a group of fused segments. Make out these regions in Metargiope and Miranda, or other spiders.

In the centipedes, and millipedes on the other hand, all of the segments are movable except a small number in the head. Examine a specimen of Scolopendra or Julus, and note that the region behind the head is flexible.

10. Phylum CHORDATA (Lat. chordatus = having a chord or cord). The most commonly known animals because they are large and conspicuous and some of them are domesticated.

Characteristics.

10*a*. Backbone composed of vertebræ (or a notochord) present. Examine skeleton of a bird, a cat, a frog, a fish, and a snake or lizard.

10*b*. Typically two pairs of jointed appendages. Recall the modifications of these appendages found in the skeletons studied in Homology. Observe also the modifications of the limbs in the skeleton of a mole; of a seal; of a porpoise; and (if available) of a snake.

10*c*. Besides the above parts there is a general internal skeleton composed of cartilage or bone.

B. SUBDIVISION OF THE PHYLA

Phyla are divided into subgroups called classes. Classes are distinguished from one another in the same way as are phyla, but by means of characters less fundamental and less primitive than those used in separating phyla. Note that this is true in the analysis of one sub-phylum, the Vertebrata, in the following exercise.

The Classes of Vertebrates

1. Class PISCES (Lat. piscis = fish).

Characteristics.

1*a*. Cold blooded, aquatic, respiring by *gills*. Observe the gills in a fish.

1*b*. Body long and pointed, and provided with *dorsal fins, tail fin, ventral fin,* and two pairs of *lateral fins*. Verify in a specimen.

1*c*. Scales cover the body; and a flap, the *operculum,* covers the gills. Verify.

2. Class AMPHIBIA (Gr. amphi = both + bios = life). Frogs, toads, salamanders, newts, etc.

Characteristics.

2*a*. Cold blooded animals usually spending part of their existence in water, part on land, capable of living either in water or on land.

2*b*. Usually possess two pairs of limbs with five digits each. Examine a toad, a frog, and a salamander for verification of, or exception to, this rule.

2*c*. Skin is without scales or other hard parts, and is slimy owing to a mucous secretion. Handle a living frog to observe these features.

2*d*. Young breathe by gills (observe a tadpole); adults usually breathe by lungs (see dissection of a frog, also the respiratory movements of a living frog).

3. Class REPTILIA (Lat. repere = to crawl). Lizards, snakes, turtles, alligators, etc.

Characteristics.

3*a*. Cold blooded.

3*b*. Skin possesses scales or hard plates. Observe in a snake; in a turtle; in a lizard.

3*c*. Body not slimy.

3*d*. Breathe by means of lungs throughout life. Note the lungs, in a dissection of the snapping turtle, or other reptile.

4. Class AVES (Lat. avis = bird).

Characteristics.

4*a* Warm blooded. How does a fowl incubate her eggs?

4*b*. Terrestrial. Even wading and swimming birds spend the major portion of their time on land.

4*c*. Body covered with feathers. Examine one or more feathers under a lens. Compare with figures.

4*d*. Fore limbs modified as wings. Examine the skeleton of a wing and note its deviations from the typical vertebrate limb. Examine also the character of the feathers which add to the wing expanse.

4*e*. Absence of teeth in modern birds. Examine a bird skull.

5. Class MAMMALIA (Lat. mamma = a breast). Man, monkeys, whales, bats, seals, and many common wild and domestic animals.

Characteristics.

5*a*. Warm blooded. What is your own temperature?

5*b*. Mostly quadrupeds. Some, however, progress on two feet (man), some by "wings" (bats).

5c. Skin covered with hair. Observe hair in squirrels, bats, or other quadrupeds; also spines in hedgehog or porcupine.

5*d*. Young nourished after birth by secretion from mammary glands of mother.

C. SUBDIVISION OF THE CLASSES

To illustrate the subdivision of the *classes* of animals into smaller groups called *orders*, the Amphibia and Reptilia may be selected. There

are but three orders of living Amphibia, and four orders of Reptilia. Note, in the following exercises, that the characters used to separate orders are less fundamenal than those used to separate classes.

The Orders of Amphibia

1. Order CAUDATA. Salamanders, newts, etc.

Characteristics.

1*a*. Tailed. See Ambystoma, Diemictylus, Cryptobranchus, Plethodon, Desmognathus, and others.

1*b*. External gills sometimes present throughout life (Siren, Necturus, Proteus), sometimes absent in the adult stage (Ambystoma, Triton, and others).

2. Order SALIENTIA. Frogs, toads.

Characteristics.

2*a*. Tailless. See Rana, Hyla, Chorophilus, Acris, Bufo.

2*b*. External gills absent in adult. See the forms listed under 2*a*.

3. Order APODA. Cœcilians.

Characteristics.

3*a*. Without limbs. See Siphonops.

3*b*. Eyes concealed. See Siphonops, and compare with any of the Salientia.

The Orders of Reptilia

1. Order TESTUDINATA. Turtles.

Characteristics.

1*a*. Body encased in a bony capsule composed of dermal plates. Observe any turtle. In a cleaned skeleton note how the shell is attached to the skeleton.

1*b*. Jaws without teeth. Examine a cleaned turtle skull.

1*c*. Quadrate bone immovable. Examine skull. The quadrate is at the angle of the upper jaw, and forms the articular surface for the attachment of the lower jaw.

1*d*. Usually five digits in each fore foot, and four or five in each hind foot. Verify in as many specimens as possible.

1*e*. Only one nasal aperture in skull. Observe in any cleaned skull.

2. Order RHYNCHOCEPHALIA. This order is represented by only one species, which is found in the New Zealand region. Owing to the rarity of the material, the internal features listed below cannot be demonstrated. A figure of the whole animal is desirable.

Characteristics.

2*a*. Vertebræ biconcave.

2*b*. Quadrate bone immovable.

2*c*. Pineal eye fairly well developed. Examine the dorsal side of the head of Sphenodon; note a whitish spot some distance back of the eyes.

2*d*. Anus a transverse slit.

3. Order CROCODILINI. Crocodiles and alligators.

Characteristics.

3*a*. Vertebraæ usually concave in front. Material for demonstration will probably not be available.

3*b*. Fore limbs bear five digits, hind limbs four. Verify in specimens.

3*c*. Anal opening a longitudinal slit. Compare with a Rhynchocephalian in this respect.

3*d*. Quadrate immovable. See alligator skull.

4. Order SQUAMATA. Snakes, lizards, and chameleons.

Characteristics.

4*a*. Vertebræ usually concave in front. Verify on specimens.

4*b*. Quadrate freely movable. See skull of snake; also of the "blind worm" Anguis. Advantage of this feature? What is the food of snakes?

4*c*. Anus a transverse slit. See Thamnophis, Bascanion, and Sistrurus or any other snake.

D. SUBDIVISION OF THE ORDERS

Orders are divided into families, on the basis of characters less fundamental than those which furnish the basis for the division of classes into orders. To illustrate the features that distinguish families, a few families of turtles[1] may be used. All the families listed below belong to one order, the Testudinata, and there are several families of this order that are not mentioned.

Some of the Families of Testudinata

The characters exhibited by turtles are as follows:

1. Neck retractile in vertical plane, or (2) bending laterally.

3. Cervical vertebræ without (or with only small) transverse processes, or (4) with strong transverse processes.

[1] To the Teacher.—It is not necessary that all of the families of turtles listed here be used in this exercise. It is suggested that the families represented in the region where the work is being done be used, and that if the teacher is more familiar with other groups than with the turtles other keys be substituted. The main requirement is that the characters used be the true *family characters* and not superficial ones which merely happen to differentiate the families of a particular area.

5. Last cervical and first body vertebræ articulated by centrum and zygapophyses, or (6) articulated by zygapophyses only.

7. Marginal bones forming a complete series, or (8) absent or in an incomplete series.

9. Squamosal and parietal bones separated, or (10) forming a suture.

11. Limbs not paddle-shaped, or (12) paddle-shaped.

13. Nuchal plate with costiform processes, or (14) without costiform processes.

15. Plastron composed of nine bones, or (16) eight bones, or (16*a*) eleven bones.

17. Caudal vertebræ mostly opisthocœlous, or (18) procœlous.

Some of the families are given below with the characters, and these families are represented by specimens. Study the specimens with the list of characters, determine the families to which they belong and describe each family in your notes.

Family Cheloniidæ.—Characters 1, 3, 5, 7, 10, 12, 14, 15, 18.
Family Trionychidæ.—Characters 1, 3, 6, 8, 9, 11, 13, 15, 18.
Family Chelydridæ.—Characters 1, 3, 5, 7, 9, 11, 13, 15, 17.
Family Pelomedusidæ.—Characters 2, 4, 5, 7, 9, 11, 14, 16*a*, 18.
Family Kinosternidæ.—Characters 1, 3, 5, 7, 9, 11, 13, 16, 18.
Family Testudinidæ.—Characters 1, 3, 5, 7, 9, 11, 14, 15, 18.

E. SUBDIVISION OF THE FAMILIES

To the Teacher.—It is expected that the teacher will at this point introduce keys to the genera and species of some one or a few groups of animals. The group selected should preferably be represented in the general region where the work is being done so that the exercise will both acquaint the student with species with which he will come in contact and with the characters used to differentiate the subdivisions of the family. The teacher should select groups with which he is most familiar and of which representative specimens can be most easily acquired. The keys may be compiled from general systematic treatises or from state monographs. As examples of the works which may be used the following may be cited:

General Systematic Treatises:

Chapman, Handbook of Birds of Eastern North America.
Bailey, Handbook of Birds of Western United States.
Dickerson, The Frog Book.
Jordan, A Manual of the Vertebrate Animals of the United States.
Walker, A Synopsis of the Classification of the Fresh-water Mollusca of North America, North of Mexico.

STATE MONOGRAPHS:

BARROWS, Michigan Bird Life.

FORBES and RICHARDSON, The Fishes of Illinois.

ORTMANN, The Crawfishes of Pennsylvania.

RUTHVEN, THOMPSON and THOMPSON, The Herpetology of Michigan.

WALKER, An Illustrated Catalogue of the Mollusca of Michigan: Part 1. Terrestrial Pulmonata.

TO THE STUDENT.—Generic characters, that is characters which permit of the breaking up of the families into groups of forms, are generally structural and less variable than the so-called specific characters by means of which the genera are in turn divided into species. The specific characters may be and generally are superficial, such as form and color of the body or its parts, but the characters differ greatly in different groups and may in fact be any difference which is sufficiently constant. As an aid in identification "keys," that is simplified tabulations of characters, are compiled by systematists. These keys do not necessarily show the actual relationships of the forms in the groups which they analyze, but they illustrate the characters used and the methods employed in analytical systematic zoology.

F. SUMMARY

State the principles at the basis of classification. What differences in degrees of relationship are expressed by groups of different rank, as orders, families, etc.? How do the characteristics of the groups show these differences? What is the relative age of groups of different ranks, as orders, families, etc.? Give the reasons for the last answer.

The summary need not directly answer these questions, but the answers should be included in the course of the discussion.

EXERCISE XI

ECOLOGY AND ADAPTATION

In this exercise will be studied two species of animal found in terrigenous bottoms of lakes, with special reference to the structures and habits which fit them for such habitat. Animal reactions will be studied in forms from other habitats. Drawings, answers to questions, and a summary should be handed in.

A. TERRIGENOUS BOTTOMS

Character of Terrigenous Bottoms

Examine photographs of portions of some lake showing (1) a considerable area of barren, sandy shoal, and (2) a photograph of a limited portion of the bottom of such a shoal. If such habitat is easily available for actual observation, this part of the work could be done in the field with considerable profit. The first two animals to be studied were taken in such a situation. Note:

1. The almost complete absence of vegetation. How is this feature accounted for?
2. The waves, showing the beach to be wind-swept. What relation does this fact bear to (1) above? On which shore of a lake might such a beach be located?
3. The sand ripples. What causes them? Relation to (1) and (2) above?
4. Flecks of foam on the surface of the water. Cause?
5. In such an environment what are the conditions with respect to (*a*) dissolved oxygen content of the water, (*b*) carbon dioxide, (*c*) decaying organic matter, (*d*) extremes of temperature as compared with the deeper water, (*e*) light, (*f*) molar agents, (*g*) materials for holdfasts, shelter, or abode.

6. By what methods can animals normally inhabiting such a situation maintain their positions there?

Fauna of Terrigenous Bottoms

(*a*) **Lampsilis or Anodonta (Fresh-water Musseis).**

1. Study living specimens in shallow dishes or in small aquaria containing water and provided with sand bottoms. Be careful *not* to *jar* them.

1*a*. Note the two *siphonal openings* with fringed borders at one end of the shell. With a pipette carefully and without touching the animal put some powdered carmine mixed with water just opposite the openings and demonstrate that water enters one (inhalent) and leaves the other (exhalent).

1*b*. Observe the large fleshy, plow-like *foot*, buried in the sand. It may be seen if the animal is near the sides of the glass dish, or demonstrated by lifting the animal quickly before it contracts.

1*c*. Make a *sketch* or diagram of a mussel from the side, showing the position of the long axis of the shell, that of the surface of the sand, the siphonal openings (with direction of the current for each shown by an arrow), and the outline of the extended foot. The lower end is the *head*, or anterior end, the upper is the *posterior* end. The dorsal surface is that bearing the *hinge* with its dark brown ligament. Make a full page sketch, since other structures are to be drawn in later.

2. Study fresh or preserved material, including some females with young (glochidia) in the gills.

2*a*. Remove the right valve of the shell by cutting against its inner surface, with a stout knife, the strong *adductor muscles*, one near each end, and pushing the mantle from the valve to be removed. Place the half of the shell containing the animal in a dissecting dish and cover with water.

2*b*. Note the *mantle* lining the other shell valve, and the mantle cavity between the two lobes of the mantle. In the mantle cavity find:

2*c*. The *gills*, two leaf-like structures on each side. Turn back the upper pair and find:

2*d*. The hard, contracted *foot* near the anterior end on the ventral side. It continues backward into the *visceral mass* which contains alimentary canal, reproductive, circulatory and excretory organs. These will not be dissected, but may be seen in charts of typical mussels.

2*e*. The *labial palps*, triangular ridged flaps, two on each side just anterior to the gills.

2*f*. The *mouth* opening between the labial palps of the two sides. Probe it with the blunt end of a needle or other instrument. *Sketch* in natural position the foregoing parts in the outline already made.

2*g*. With dissecting microscope examine the surface of a living gill and note the numerous small openings leading into its interior. With compound microscope observe the cilia which cause water currents to pass in through the openings. These

can best be seen in a portion of a single *lamella* (half of one gill) mounted in Ringer's solution between slide and cover-glass, and studied with high magnification. *Sketch* a little of the gill surface showing the cilia and their relation to the openings.

2*h*. Tear apart the two lamellæ of which each gill is composed, and note that these enclose vertical tubes which extend from the free edge of the gill to its attached dorsal edge. With scissors cut thick cross-sections of the gill to show the lamellæ and tubes. *Draw* a small portion of the cut edge.

2*i*. Put a probe into the exhalent siphonal opening. It enters a channel above the attached edge of the gills. Cut through the gills by drawing a knife along the probe, and explore the gill chamber into which the vertical tubes from the gills open.

2*j*. Trace the course of the water from the inhalent siphon into the mantle cavity, thence through the gill tubes, and out at the exhalent opening.

2*k*. How do you suppose the animal breathes? Gets its food? Removes the waste products of respiration, digestion, and excretion?

2*l*. Examine a specimen containing glochidia and note how the expanded spaces between the gill lamellæ serve as brood pouches.

2*m*. Examine some of the glochidia of Anodonta in water under a low magnification. *Sketch* to show:

1. The triangular valves of the shell.
2. The large tooth at the apex of each valve.
3. The strong *adductor* muscle.
4. The thread-like *byssus* (of uncertain function).

2*n*. By reading one of the following references, or by consulting the instructor, learn how the glochidia attach themselves to fish and are distributed by them.

Lefevre, G. and Curtis, W. C., 1912. Studies on the Reproduction and Artificial Propagation of Fresh-water Mussels. Bull. Bureau of Fisheries, 30: 107–201.

Needham, J. G. and Lloyd, J. T., 1916. The Life of Inland Waters. Comstock Publ. Co. See pp. 287–292.

Baker, F. C., 1916. The Relation of Mollusks to Fish in Oneida Lake, Tech. Publ. No. 4., N. Y. State College of Forestry at Syracuse University. See pp. 219–223.

2*o*. Examine the demonstration of towings made with a fine mesh Birge cone net from the sandy shoal habitat where the mussels were collected. In general, what kinds of minute organisms occur there? Then:

2*p*. Examine (demonstration) contents of the anterior part of the digestive tract. Can you recognize any of the organisms observed in 2*o*? Approximately what proportion of the contents is composed of organisms? If other materials are present, what are they and what is their source? By what means are the food particles brought to the mouth?

3. The mussel must maintain an upright position by means of the foot, and have its siphonal openings uncovered in order to breathe and feed. On what kind of a bottom would it thrive best? Why is it not found on the ooze bottom in deep water far from shore? Why does it not occur on a solid, clean swept rock bottom? What other animals must live in the same body of water with it? In short, in what situations would you be most likely to find fresh-water mussels and why?

(*b*) **The Nymph of a Gomphine Dragon-fly (Gomphus).**

1. Examine living specimens in a dish of water on sand bottom. Note:

1*a*. The tube-like tip of the abdomen with its open end thrust up through the sand. Water enters this opening (anus) to reach the gills which are contained in a rectal respiratory chamber. The opening is guarded by an elaborate strainer.

1*b*. That the animal burrows through the sand when disturbed. Note if possible the method of burrowing.

2. Examine a specimen in a watch glass of water under a dissecting microscope and note:

2*a*. The large grasping *labium* or lower lip with its hooks and spines. This is hinged so that it may be extended forward far beyond the head, and is capable of being thrust forward and withdrawn with great rapidity. Pull it forward with forceps. The animal is carnivorous and predatory.

2*b*. The adaptations for digging:

1. The flattened head.
2. The flattened fore legs thrust forward.
3. The remaining legs pressed against the side of the body where they are out of the way; the hind pair extended backward against the side of the abdomen.

3. How does the form of the head and legs, and the structure of the respiratory organs adapt the animal to burrowing in the soft bottom?

B. ANIMAL REACTIONS

Of importance in determining the habitat of animals is the manner in which they react to different factors in their environment. A few reactions will be observed here.

1. Place a number of living planarians (Planaria sp.) in each of several

shallow dishes containing water and a moderate amount of algæ or a few small pieces of water weed (Elodea). Allow these dishes to stand for some time absolutely undisturbed and add no food. Where are the animals? Why? What is the stimulus involved? As gently as possible, place a small, recently excised portion of the body of an earthworm just below the surface of the water. Watch the dish intently for signs of activity on the part of the planarians. What kind of activity is manifested? What is the end result of this activity? To what kind of stimulus is it a response? Is it positive (going toward the source of the stimulus) or negative (going in the reverse direction)? Of the stimuli referred to above, which is the stronger? Evidences?

2. Observe land sow-bugs (Porcellio sp.) in a petrie dish half of which is covered with black paper to exclude the light, leaving the other half well lighted. Ten sow-bugs have been placed in this dish and left undisturbed so that they might come to rest. What is the distribution of the animals and how do you account for it? What is the stimulus involved and how do they react to it?

3. Observe ten land sow-bugs in another petrie dish half of which contains loosely laid thin sheets of mica, the other half being clear. In which half are most of the sow-bugs? Which of the following factors, if any, are they reacting to: light, gravity, contact, moisture, temperature? Reactions to these are called respectively, phototaxis, geotaxis, thigmotaxis, hydrotaxis, and thermotaxis.

4. In a pan half of the bottom of which is covered with rather moist soil and half with dry soil note the reactions to moisture in this species of land sow-bugs.

Make records of observations. In what sort of environment would you expect to find land sow-bugs? Do you conceive the reactions of these animals to be advantageous to them?

C. SUMMARY

How are the animals studied in this exercise adapted to their environment? Is the adaptation morphological, physiological, or both? Examples. Have you witnessed any adaptation to the biological environment (the other organisms in the vicinity)? How may animals have become adapted to their environment? (Give alternative views if you can.) What is ecology?

These questions are suggestive only, and are not intended to indicate the order in which topics are to be discussed in the summary, nor to limit the summary to these topics.

EXERCISE XII

ZOOGEOGRAPHY

The laboratory exercises in Zoogeography will be limited to the conditions in North America. Their object is to develop a general knowledge of the environmental conditions in North America, and their relations to the ranges of animals. Vertebrates are principally used because the ranges are better known. The maps should be carefully and neatly prepared as otherwise their significance will be obscured.

A. GENERAL AREAS OF ENVIRONMENTAL CONDITIONS IN NORTH AMERICA

North America may be divided into several regions which have characteristic physical conditions. These areas support characteristic floras which provide an easy means of establishing their boundaries. It should be kept in mind that the boundaries of the different regions are sharply drawn only at the seashore; where the regions come together inland there is in every case a zone of transition due to the fact that the environmental conditions change gradually and not suddenly.

1. On an outline map of North America indicate by shading or symbols the location of the areas covered by the following floras: coniferous forests, deciduous forests, prairies, plains and deserts. The map in "Principles of Animal Biology" (Shull, LaRue and Ruthven), Chapter XIV, may be followed in preparing this map.

2. Compare the map just made with the map of the regions in eastern United States given in "Principles of Animal Biology" and note the subregions into which general regions may be divided. Note the transition areas.

B. DISTRIBUTION OF SOME TYPICAL ANIMALS OF NORTH AMERICA

3. Plot upon outline maps of North America the ranges of several exclusively terrestrial animals. Any of the following forms are suitable.

3*a*. Two of the garter snakes, *Thamnophis radix* and *Thamnophis butleri*. Their ranges[1] are described by Ruthven, Bull. U. S. Nat. Museum, No. 61. Plot the ranges of both species on one map.

[1] The maps from these books may be duplicated and furnished to the students in sufficient numbers.

3*b*. The American Bison and the Moose, on one map. (From Seton, Life Histories of Northern Animals).

3*c*. The Willow Ptarmigan, White-winged Cross-bill, and Road Runner on one map. (From Chapman, Handbook of the Birds of Eastern North America, and Bailey, Handbook of the Birds of Western United States. These books contain descriptions, not maps, of ranges.)[1]

4. Plot upon outline maps of North America the distribution of one or more semi-aquatic species. The North American minks are suggested for this purpose (Seton, Life Histories of Northern Animals).

5. Compare the distribution maps which you have made with the map showing the natural regions in North America as indicated by the dominant vegetation and summarize the distribution of the species in terms of natural regions and geographic location. (*Example:* The range of species X is the coniferous forest region in eastern North America from the Atlantic coast to the 100th meridian and from Hudson Bay on the north to Lake Superior on the south.)

In which cases, if any, does the range approximately correspond to the distribution of certain types of vegetation? (See map prepared in paragraph 1 above.) In which cases, if any, does the range bear no relation to the vegetation areas? Explain the difference in the two cases.

It will be noted that the ranges of the animals do not correspond exactly to the natural regions. There are several reasons for this, such as incomplete knowledge of the extent of the range, too general summaries of the distribution, the fact that the regions merge gradually into each other or interdigitate where they come in contact, and the different effects of the environments upon different animals.

The intermediate regions are characterized to some extent by intermediate conditions, but at least frequently the environments interdigitate. For an example of the latter phenomenon consult "Principles of Animal Biology," by Shull, LaRue and Ruthven.

C. ADAPTATIONS OF ANIMALS TO THE CONDITIONS IN THE REGIONS WHICH THEY INHABIT

As brought out in the exercise on ecology animals are adapted to the conditions in which they live. It follows from this that a difference in any conditions of the environment may serve to limit the distribution of a form. Owing to the complexity of the relationship between animals and their surroundings it is difficult to determine the exact factor or factors restricting the distribution of a given species at a given point, but certain very general adaptations may be easily recognized.

[1] Other ranges of terrestrial animals may be substituted for the ones mentioned, or used in addition to them.

6. Observe the following structural adaptations of animals:

6*a*. The locomotor appendages of a fish; two turtles, for example, *Chrysemys marginata* and *Gopherus polyphemus;* three birds, for example, a heron, a duck and a woodpecker; and two mammals preferably a mole and a flying squirrel or a bat.

6*b*. The pelage of the northern form and the southern form of the woodchuck.[1]

6*c*. The color of a forest and a desert species of horned lark.

6*d*. The molar teeth of a grazing animal (bison or cow) and a browsing mammal (the elk).

6*e*. The beaks of a duck, a woodpecker and a heron.

List the above-mentioned forms with the environmental conditions in which the structures fit them to live and the changes in conditions which would probably destroy the usefulness of the structures and therefore limit the distribution of the animals.

D. SUMMARY

Discuss briefly the relation of diverse environmental conditions to the distribution of animals.

[1] Certain other animals may be used equally well to show these regional differences.

EXERCISE XIII

PALEONTOLOGY

In the exercise on Homology it was found that the limbs of vertebrates begin their development in the same way, as a simple outpushing of the body wall, whereas the adult limbs of different vertebrates are quite unlike in the details of structure. These and other facts are believed to show that all vertebrate animals have descended from a common ancestor. If this belief is well founded, vertebrates have changed ("evolved") greatly in the generations subsequent to the common ancestor.

In the following exercises it is shown in the case of two typical groups of animals, one vertebrate and one invertebrate, that such an evolution has actually taken place. Either or both of these exercises may be used at the option of the instructor. The change is demonstrated by the remains of animals preserved in the rocks as fossils. In general, the deeper rock strata contain the fossils of the more ancient animals, the more superficial rocks the more recent animals. Why?

In the study of the fossils used, reference should be made to the geological time scale in "Principles of Animal Biology," by Shull, LaRue and Ruthven, Chapter XV, or in Pirsson and Schuchert's "Text-book of Geology." This time scale should be before the student throughout the exercise.

A. EVOLUTION OF THE CEPHALOPODA

The cephalopods of the past lived *within* their shells, like the present day Nautilus, but unlike the squid or cuttlefish, which are also cephalopods.

Examine a bisected shell of Nautilus, also a shell of Nautilus containing the animal. Note that the shell is divided into a number of chambers by *septa* (singular, *septum*). These were successively produced from the center to the opening of the shell. The animal, as it grew, moved forward in its shell at intervals, and formed new septa behind it.

The ancient cephalopods lived in shells somewhat similar to that of Nautilus. The line of union of a septum with the outer wall of the shell is called a *suture*. The sutures of Nautilus are not visible externally because of a pearly layer, the *nacre*, on the outside. Fossil cephalopod shells, however, usually show these sutures. Examine a fossil Loxoceras, Orthoceras, or other orthocone.

Notes.—No preliminary notes on the cephalopods are required. The questions asked below are intended chiefly to direct attention. The

drawings, and the summary (directions for which are given below), will answer most of them.

1. Study an orthocone (Loxoceras or Orthoceras, for example). This type of cephalopod was particularly common in the Ordovician and Silurian periods. What is the form of the shell? The form of the sutures? The specimen is usually only a fragment of the entire shell. Thus, in the Museum of Geology at the University of Michigan is a fragment of an orthocone 6½ inches in diameter at its larger end, 4½ inches in diameter at its smaller end. This fragment is 18 inches long. If the piece were completed at its smaller end, how long would it be? Since the animal lived only in the undivided chamber at the larger end of the shell, the shell was many times larger than its occupant.

Modern cephalopods progress backward by means of the siphon. (Examine the siphon of a squid and understand its operation.) How would the long shell affect the animal's movements?

Draw an orthocone, giving its name, to show the form of the shell and of the sutures. A line drawing is sufficient but should be carefully made.

2. Examine a gomphoceran (Poterioceras or some other). These forms are recovered from the Ordovician to the Carboniferous periods. What is the shape of the shell? Form of the sutures? How much of the shell was occupied by the animal? Is this shell more cumbersome, or less so than that of the orthocone?

Draw a gomphoceran carefully (line drawing).

3. In a nautiloid (Eutrephoceras is an example), what is the form of the shell? Of the sutures? Examine a bisected fossil nautiloid, if one is available, noting the form of the septa; compare it with the bisected shell of the modern Nautilus. The nautiloids were most abundant in Silurian and Devonian times, though some survived those periods, and one of them, the pearly Nautilus, is still living.

Draw a nautiloid, showing all the visible sutures.

4. Compare the shell of a goniatite (Aganides or some other) with those of the preceding forms, particularly the nautiloid. Note the form of the sutures. Goniatites were most abundant in Carboniferous times (see time scale).

Draw a goniatite, being careful to represent all the sutures in their correct form.

5. Study a ceratite (Ceratites or any other). What is the form of the suture? How do the sutures compare in complexity with those of a goniatite? The ceratites were largely Triassic.

Draw a ceratite to show its sutures.

6. Study an ammonite (Scaphites or any other). These reached their climax in the Jurassic to the Cretaceous periods. The sutures are very crooked fine lines on the surface. Do not confuse the coarse ridges on the surface with them. Trace very carefully at least one suture

completely around one coil of the shell before attempting a drawing. The chances for error are large, because adjoining sutures approach one another very closely at various points. Examine other ammonites if possible.

Draw an ammonite at least natural size, showing two of the sutures. The latter should be very accurate pictures of the specimen used, not merely a diagrammatic representation of the *kind* of sutures found in ammonites in general.

B. EVOLUTION OF THE HORSE

The development of the horse, as far as known from fossils, took place entirely in Tertiary time. The undiscovered ancestor was undoubtedly a small animal, with five toes on each foot, and nails instead of hoofs. In the following exercise the evolution of the horse will be traced with respect to (1) number of toes, (2) form of teeth, and (3) size of body and skull.

Many of the specimens used in the laboratory are casts of fossils, and *must be handled with care.* The student should see some of the actual fossils also, if these are available.[1]

The Feet.

1. Examine casts of the bones of the fore and hind feet of Eohippus or Orohippus. They are of natural size. How many digits in each foot? Are any of the digits distinctly shorter than the rest?

From the wall chart note the geological age to which these forms belong. *Draw* both fore and hind foot, representing the proportions with care, and carefully distinguishing the individual bones. Indicate by Roman numerals which of the ancestral five digits are left (see Shull, LaRue and Ruthven, "Principles of Animal Biology," Chapter XV). The figure may be less than natural size.

2. Study a cast of the foot of Mesohippus. How many digits? Which ones? What is the relative size of the various digits? Compare in size with Eohippus or Orohippus.

To what geological period does Mesohippus belong? *Draw* the foot of Mesohippus with care, indicating which digits are present.

3. Examine the fore foot of Hypohippus. Compare in size with the foot of Mesohippus. Note the size of the third digit as compared with the second and fourth. Is the third digit relatively larger, or relatively smaller, than in Mesohippus? Did the lateral digits of Hypohippus reach the ground? Observe the nodules at the back of the metacarpals

[1] Other genera of similar nature may be substituted for the ones named below. Hypohippus and Hipparion, which appear not to be in the direct line of evolution, may be omitted if desired.

at their proximal end. What do they represent? Which nodule is the larger? Does this relative size signify anything?

In what geological time did Hypohippus exist?

4. Study the fore or hind foot of Merychippus. Compare in length with the fore foot of Hypohippus. Did the lateral toes reach the ground? Are there any indications of the first and fifth digits (cf. Hypohippus)? How recent is Merychippus?

Draw a foot of either Hypohippus or Merychippus. If Hypohippus is selected for this figure, view it obliquely from the side so as to include the vestige of one of the lateral metacarpals. Represent the individual bones carefully in their proper proportions.

5. Foot of Hipparion or Pliohippus. Compare in height with Merychippus. How well developed are the second and fourth digits? Compare with Hypohippus and Merychippus.

Geological period?

6. Equus, fore or hind foot, either fossil or modern. Compare in size with the earlier forms. Look for vestiges of the second and fourth digits.

Draw the fore or hind foot of Hipparion or Pliohippus or Equus with care. Turn in such a position as to show one splint bone.

The Teeth and Skull.

1. Examine the skull of Eohippus. Note size of the jaws. Note position of orbit of eye relative to teeth. Ask for a specimen, photograph or cast of a tooth of Eohippus. What is the relative length of the crown and the roots? (Note whether the roots are entire or not). Observe the tuberculate surface of the tooth (that is, the cusps or prominences on it).

2. Study the skull of Mesohippus. Compare with Eohippus. Where is the orbit relative to the teeth? A fossil tooth, photograph or cast will be furnished. What is the relative length of crown and root? What is the nature of the surface? *Draw* the tooth of Mesohippus, either from the original or from a cast or photograph, showing as accurately as possible (*a*) the length of crown and root, and (*b*) the form of the upper surface. Shading is desirable to show the latter feature. View the tooth obliquely so as to include roots and upper surface in one figure.

3. In specimens, casts, or photographs of the tooth and skull of Merychippus, note (*a*) the size of jaw, (*b*) position of orbit, (*c*) the size of the crown of the tooth, (*d*) the character of the surface of the tooth. *Draw* the tooth of Merychippus or copy the photograph in a line drawing.

4. Compare the skull and teeth of Equus (either fossil or modern) with the preceding forms. Examine a bisected tooth and note the extent of the pulp cavity. *Draw* the tooth of Equus in oblique view to show roots and upper surface in one figure.

Size of Body.

1. From the casts or fossils of the feet, note the increase in size through successive geological periods.

2. On a chart representing restorations of the entire animals, based on measurements of the fossil bones, note the increase in stature from Eohippus to Equus.

C. SUMMARY

State carefully the course of evolution of the cephalopods and of the horse with regard to the features studied in the foregoing exercise. Make reference to your figures. Note that some of the forms studied may not be in the direct line of descent, but are probably offshoots. Which are these? In which continents did the early, middle, and late development of the horse chiefly take place? Make use of these points in your summary. The summary of the horse should include a table showing the continents where its development took place, the geological periods, and the changes in feet, teeth, skull, and stature.

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2. Lull, R. S., "Evolution of the Horse Family," American Journal of Science, March, 1907.
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4. Dendy, A., "Outlines of Evolutionary Biology," pp. 307–312.
5. Cope, E. D., "Primary Factors of Evolution," pp. 146–149.
6. Matthew, W. D., "The Evolution of the Horse," supplement to American Museum Journal, January, 1903.

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1. Lull, R. S., "Organic Evolution," Chapter XXVI, especially pp. 429–433.
2. Williams, H. S., "Geological Biology," Chapter XIX, especially pp. 350–358.

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